IMMUNOGLOBULIN ISOTYPE RESPONSE OF *FASCIOLA SPP.* INFECTED SHEEP AND CATTLE TO DEFINED *FASCIOLA SPP.* ANTIGENS.

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This thesis is dedicated to:

My late Mother Delia Y. Banda (1933-1989) & my son Elias I. K. Phiri, my daughter Lwindi I. K. Phiri and my Wife, Arlene C. Phiri

CONTENTS

LIST OF ABBREVIATIONS AND SYMBOLS

γ-GT	γ-glutamyl transferase
μg	Microgram
μl	Microlitre
^{35}S	A radioactive isotope of sulphur
ACU	Association of Commonwealth Universities
β-HOB	β-Hydroxylbutyrate
BBS	borate buffered saline
E/S	Excretory or secretory products
ELISA	Enzyme linked immunosorbent assay
g	Relative centrifugal force
GLDH	Glutamate dehydrogenase
GST	glutathione S-transferase
Hb	Haemoglobin
IgA	immunoglobulin isotype A
IgE	immunoglobulin isotype E
IgG	total immunoglobulin
IgG_1	immunoglobulin isotype G_1
IgG_2	immunoglobulin isotype G_2
IgM	immunoglobulin isotype M
kDa	Kilodalton
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
ml	millilitre
mm	millimetre
mM	millimole
NEJs	newly excysted juveniles
NGS	Normal goat serum
nm	nanometre
NOG	n-octyl glucopyranoside
NRS	Normal rabbit serum
°C	Degrees centigrade
OD	optical density
P	Probability
PBS	phosphate buffered saline
PCV	Packed cell volume(s)
RBC	Red blood cells
SDS-PAGE	Sodium dodecyl sulphate polyacryl amide gel electrophoresis
v/v	Volume by volume
w/v	Weight by volume
WBC	White blood cells
wpi	Weeks Post infection

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SUMMARY

Following the general introduction, chapter one, the thesis is divided into four chapters, covering literature review, materials and methods, results and discussion and conclusion.

Fasciolosis is a liver fluke disease, caused by *Fasciola hepatica* in temperate regions and high altitude areas of the tropics and subtropics and by *Fasciola gigantica*, which is restricted to the tropics and subtropics. Liver flukes have a wide range of definitive hosts, including man and in particular domestic ruminants, but the various hosts are known to differ greatly in their resistance to infection with these parasites. For example sheep are considered susceptible to challenge infection while cattle develop resistance.

F. hepatica secretes the enzyme cathepsin-L1 protease (Fh-cathepsin) which has a molecular weight (MWt) of 27 kDa. It is considered to have a functional role in parasite evasion of the host immune response, through cleavage of host immunoglobulin. The enzyme, glutathione s-transferase (GST) is of 27.8-29 kDa MWt, is also secreted by *F. hepatica* (Fh-GST) and is thought to be involved in the detoxification of exogenous (xenobiotic) and endogenous derived toxic compounds. Both enzymes form part of the fluke excretory/secretory (E/S) products and are of interest as they are considered as vaccine candidates against fasciolosis.

This study investigated the immunoglobulin isotype responses of sheep and cattle, chronically infected with *F. hepatica* and *F. gigantica,* to defined the fluke antigens (*F. hepatica* E/S products (Fh-E/S) or *F. gigantica* E/S products (Fg-E/S), Fh-

cathepsin and Fh-GST). It was decided to study the immune response in chronically infected animals since immunity is considered to play a potentially more important role in chronic infection, than in acute infection, which is characterised by the death of the animal through anaemia and blood loss caused by the migrating flukes. Serum and faecal samples were collected weekly while the severity of the infections were defined using clinical, parasitological, haematological, biochemical and pathological parameters.

Serum and faecal antibody (total Ig, IgG_1, IgM, IgG_2 and IgA) responses to 24-48 hour Fh-E/S and Fg-E/S, adult Fh-cathepsin and adult Fh-GST were determined by indirect Enzyme-linked Immunosorbent Assay (ELISA). The antigen recognition profile of the *Fasciola* spp. infected sheep and cattle to Fh-E/S and Fg-E/S was examined by sequential Western blotting.

The general clinical and pathological pattern, combined with the parasitological, biochemical and haematological data confirmed that, in the main, the sheep and cattle were suffering from chronic fasciolosis. Lesions were most severe in sheep culled 11 to 20 weeks post infection (wpi) and calves with single infection, but culled 12 wpi, and those calves with challenge infection. Overall, the calves had very light infection and as a result they displayed a less severe chronic fasciolosis than that observed in sheep.

A reduction in serum glucose levels was detected in *F. hepatica* and *F. gigantica* infected sheep from 3 wpi, especially in severely infected sheep. In contrast, there was an increase in serum β-hydroxybutyrate (β-HOB) levels in infected sheep, from about 6 to 15 wpi. And the extent of the rise in β-HOB levels was associated with the severity of the infection. There was no serum glucose or β-HOB changes observed in infected

calves. This was considered to be due to the very light fluke infection. Thus, it would appear from the serum glucose and β-HOB levels, that fasciolosis leads to energy deficiency (low glucose) and ketosis (increased b-HOB) especially noticeable in more heavily infected animals.

There was an early (2-3 wpi) total Ig response to Fh-E/S and Fg-E/S, Fh-cathepsin and Fh-GST in both *F. hepatica* infected sheep and cattle. Although there was an early (2-3 wpi) total Ig response to Fh-E/S and Fg-E/S, and Fh-GST by *F. gigantica* infected animals, there was a slight delay (7 wpi) noted in the response to Fh-cathepsin. The pattern of the IgG_1 response of cattle and sheep to these defined fluke antigens was similar to that of total Ig. In fact the serum isotype response was predominantly IgG_1. The IgM response to Fh-E/S and Fg-E/S, Fh-cathepsin and Fh-GST was early in both species. In cattle the IgG_2 and IgA responses to Fh-E/S and Fg-E/S were late and more pronounced (11 wpi and 19 wpi respectively) in contrast to sheep (2 wpi for both isotypes). The serum IgG_2 and IgA isotype responses to Fh-cathepsin and Fh-GST followed the same pattern in cattle, however in sheep, responses to Fh-cathepsin was much less marked and a response to Fh-GST was not detected. A rise in total Ig and IgG_1 responses to Fh-E/S and Fg-E/S and Fh-cathepsin were detected following challenge infection in calves, but there was no increase in the response to Fh-GST. The dominance of the IgG_1 response in *Fasciola spp.* infected sheep and cattle suggests an associated Th_2 response in both species. The late IgG_2 response in cattle may suggest late Th_1 involvement in bovine cellular responses to Fh-E/S and Fg-E/S products.

The detection of serum antibody responses to Fh-cathepsin and Fh-GST in *F. gigantica* infected sheep and cattle confirmed antigenic cross-reactivity. However this

cross-reactivity may be only partial, as suggested by the late total Ig and IgG_1 response to Fh-cathepsin by *F. gigantica* infected sheep and cattle. Comparison of the isotype responses to Fh-E/S and Fg-E/S products, Fh-cathepsin and Fh-GST suggest that there is very little difference between the response to Fh-E/S, Fg-E/S and Fh-cathepsin, however, there was a relatively poor response to Fh-GST in both sheep and cattle.

Western blot analysis of *F. hepatica* infected sheep serum identified antigens of 14 kDa from 2 wpi and 54, 79 and 134 kDa MW recognised later. In *F. gigantica* infected sheep antigens of 14, 88 and 152 kDa were identified from 7-9 wpi. There was a clear shift, at patency, in the antigen recognition pattern of cattle from higher (134 kDa for *F. gigantica* infection and 142 kDa for *F. hepatica* infection) to lower (60 kDa for both parasite species) but there was no clear antigenic shift observed in sheep. None of these antigenic molecules represented Fh-cathepsin (27 kDa MWt) or Fh-GST (27.9-29 kDa MWt) used as antigens in the ELISA assay. The lower protein concentration of the E/S products used in this assay is, one possible reason for failure to detect these molecules.

There was no faecal antibody response detected in cattle to any of the three defined antigens. This might have been due either to the light infections observed in cattle or to the larger volume of faecal material produced by cattle (i.e. dilution). There was an early (2 wpi) faecal total Ig response to Fh-E/S and Fg-E/S, Fh-cathepsin and Fh-GST in *F. hepatica* and *F. gigantica* infected sheep. In fact there was no difference in faecal antibody responses to the different antigens by either *F. hepatica* or *F. gigantica* infected sheep. The isotype response was mainly IgA while a slight IgG_2 response could be detected in *F. hepatica* infected sheep. The total Ig and IgA responses

to all three defined antigens in *F. hepatica* sheep was biphasic, in *F. gigantica* infection however the phases were less defined. The 1st phase (about 2-10 wpi) is considered a response to juvenile flukes antigens after oral infection and the second peak (13-17 wpi) is considered to be in response to antigens released by adult flukes in the bile duct.

This study indicates that in serum, IgG_1 isotype responses predominate and in faeces IgA isotype responses to the three defined antigens predominate, suggesting preferentially stimulation of the Th_2 T-cell subset in sheep and cattle fasciolosis. The late IgG_2 response to *F. hepatica* and *F. gigantica* E/S and Fh-cathepsin in cattle may indicate a delayed Th_1 stimulation.

In order to elucidate the different immune mechanisms operating in infected sheep and cattle there is need for more work on the cellular responses to *Fasciola* spp. as these may be related to resistance. There was antigenic shift in cattle at patency but not in sheep. The 60 kDa molecule being recognised by cattle after the antigenic shift. It is possible that this factor may be related to acquisition of resistance by cattle to secondary *Fasciola* spp. infection

Finally the findings relating to the glucose and b- HOB levels in infected sheep may prove helpful in experiments involved in the interaction between fasciolosis and the nutritional levels of *Fasciola* spp infected animals, of particular importance in young growing animals.

CHAPTER ONE

INTRODUCTION

Fasciolosis, also known as liver fluke disease, is caused by several species of digenic trematodes belonging to the *genera Fasciola* and *Fascioloides*. The two most important species, are *Fasciola hepatica* and *Fasciola gigantica*. *F. hepatica* is more commonly found in temperate areas and at high altitude in the tropics and subtropics, while *F. gigantica*, which is more pathogenic, is found in tropical regions (Urquhart, Armour, Duncan, Dunn and Jennings, 1996).

The major definitive hosts of these parasites are domestic ruminants (cattle, sheep, goats and buffaloes) while equines (horses, donkeys, mules) are less susceptible. Pigs are considered naturally resistant to *Fasciola* infection (Boray, 1969 and Soulsby, 1982). Wild mammals may also be infected with *Fasciola* spp. and this plays an important epidemiological role (Hammond, 1972). Fasciolosis is one of the most economically important helminth diseases, hampering the productivity of domestic ruminants in endemic areas. It is also of zoonotic importance as man can be infected with adult flukes.

There are clear differences observed in acquired immunity among the various species of definitive host of *Fasciola* spp. For example, sheep show little resistance to reinfection in comparison to cattle (Boray, 1967 and Losos, 1986). Haroun and Hillyer (1986), in a review, classified cattle, rats and goats as having significant resistance to *Fasciola* spp. reinfection and sheep and rabbits as having no resistance.

During their migration in the definitive host, liver flukes excrete and/or secrete (E/S) many proteins including Cathepsin cystein proteases (Dalton and Heffernan, 1989) and Glutathione S-Transferase (Creaney, Wijffels, Sexton, Sandaman, Spithill and Parsons, 1995). One of these proteases, cathepsin-L1 protease, has been isolated from adult *F. hepatica* (Fh-cathepsin) and is of 27 kDa molecular weight (Smith, Dowd, McGonigle, Keegan, Brennan, Trudget and Dalton, 1993). Fh-cathepsin is thought to play an immunological role assisting the parasite in penetrating the gut wall and liver of its mammalian host before taking up residence in the bile ducts (Smith, Dowd, Heffernan, Robertson and Dalton, 1993). Adult *F. hepatica* Glutathione S-Transferase (Fh-GST) has also been isolated and is of 28-27.8 kDa molecular weight (Hillyer, Soler Der Galanes and Battisti, 1992). Helminth GST's are known to conjugate with other endogenous substances such as lipid hydroperoxides thereby protecting the parasite from host toxins and are also thought play a role in protecting parasite against radicals produced by the host (Brophy and Barrett, 1990).

The major immunoglobulins of cattle and sheep, IgM, IgG_1, IgG_2, IgA and IgE have been well documented as far as their structure and antigenic properties are concerned (Butler, Winter and Wagner, 1971; Tizard, 1992), but much less is known about their specific activities against pathogens, especially helminths parasites (Butler, Winter and Wagner, 1971; Tizard, 1992). Thus the involvement of the various immunoglobulin isotypes in the response of cattle and sheep to *Fasciola* spp. infection have not yet been fully elucidated. The available information, concerning isotype responses in cattle and sheep fasciolosis covers mainly *F. hepatica* infections

and responses to relatively undefined antigens such as E/S (Oldham, 1985; Chauvin, Bouvet and Boulard, 1995) or whole fluke extracts (Brown, Davis, Dobbeleare and Rice-Ficht, 1994; Clery, Torgerson, and Mulcahy, 1996). Relatively little is known about isotype responses of cattle and sheep in *F. gigantica* infection to E/S antigen or to Fh-cathepsin and Fh-GST.

A knowledge of serum and faecal isotype responses to E/S, Fh-cathepsin and Fh-GST in cattle and sheep may help in the understanding of related cellular responses and hence protective immune mechanisms. For example IgG_1 responses are associated with stimulation of Th_2 lymphocytes as has been reported in murine schistosomiasis (Mountford, Fisher and Wilson, 1994) and in *F. hepatica* chronically infected cattle (Clery, Torgerson and Mulcahy, 1996). Of the many domestic ruminants, which are infected by fasciolosis, sheep and cattle were selected for this study because of their importance as domestic animals and also because they differ in both their response and resistance to fasciolosis.

These experiments were therefore designed to study sera and faecal immunoglobulin isotype responses of *F. hepatica* and *F. gigantica* infections in cattle and sheep against *Fasciola* spp. E/S, Fh-cathepsin and Fh-GST.

Chronic experimental infections in sheep and cattle were established and the severity of these infections defined by clinical, parasitological, pathological, haematological and biochemical indicators. It was decided to study the immune response in chronically infected animals since immunity was considered to play potentially a more important role in chronic infection than in acute infection, where the primary cause of death is anaemia and blood loss caused by migrating flukes.

The objectives of this study were:

1. To standardise indirect Enzyme-Linked Immunosorbent Assays (ELISA) designed to detect total Ig, IgM, IgG_1, IgG_2 and IgA isotype responses in *F. hepatica* and *F. gigantica* infected sheep and cattle to 24-48 hour adult *Fasciola* spp. E/S, adult Fh-cathepsin and adult Fh-GST.

2. To sequentially monitor the serum and faecal immunoglobulin isotype responses of sheep and cattle chronically infected with either *F. hepatica* or *F. gigantica* to these defined antigens.

3. To examine the isotype responses in order to try to associate these with possible T-cell involvement in the response to fasciolosis. By comparing sheep (susceptible) and cattle (resistant) an understanding may be reached of the immune mechanisms contributing to resistance in cattle.

4. To compare this serological pattern with the original infection dose, fluke recovery, pathology and biochemistry including glucose and β-hydroxybutyrate serum levels. These last parameters were studied in order to examine the effects fasciolosis has on the metabolism of carbohydrates and volatile fatty acids and their contribution to ketosis (ketone bodies accumulation) in sheep and cattle. The liver, which stores glycogen, the major source of glucose, is severely affected during fasciolosis in ruminants.

CHAPTER TWO

LITERATURE REVIEW

2.1 AETIOLOGY

2.1.1 Causative Organism

Hepatic fasciolosis or liver fluke disease are terms commonly used to describe the disease caused by flukes belonging to the genus *Fasciola* (Malek, 1980; Losos, 1986; Hammond and Sewell, 1990; Bürger, 1992). However clinicians and pathologists consider liver fluke disease as that caused by digenean trematodes of the genera *Fasciola, Fascioloides* and *Dicrocoelium* (Jones and Hunt 1983; Urquhart, Armour, Duncan, Dunn and Jennings, 1987; Blood and Radostits, 1989). The most important species of these genera are *Fasciola hepatica* (the common liver fluke) *Fasciola gigantica* (the large African liver fluke), *Fascioloides magna* (the large liver fluke) and *Dicrocoelium dendriticum* and *D. hospes* (the lancet flukes) respectively. But world wide it is *F. hepatica* and *F. gigantica* that cause major production loss.

2.1.2 Taxonomy

The members of the Phylum *Platyhelminthes* were first described by Rudolphi "the father of parasitology" in 1806. Ershov (1960) and Georgi (1985) described them as soft-boded, flattened dorsoventrally and hermaphroditic. Liver flukes are members of the Order *Digenea* because they undergo an indirect development with sexual and asexual generations parasitizing alternate hosts (Ershov

1960). Many authors, including Kendall and Parfitt (1959), believe that only two species of the genus *Fasciola* find a world wide distribution namely the temperate *Fasciola hepatica* and its tropical and subtropical relative *Fasciola gigantica*. Varma (1953) proposed a new species *Fasciola indica* on the basis of apparent morphological differences between fixed material from Africa and Asia and after examining a small sample of eggs .

Watanabe (1962) supported Varma's proposal after studying some biological and morphological aspects of liver flukes in Japan. Kendall and Parfitt (1959), however, were unable to find any morphological or biological differences after studying African and Pakistani liver flukes and eggs. Kendall (1965) considered *F. indica* to be synonomous with *F. gigantica* any minor differences being minor *intra* species variations. Blair and McManus (1989) after studying the rDNA restriction map of the Japanese species concurred with Kendall's (1965) observations.

There appears to be very little disagreement about the *Fasciola* species present on the Africa continent. Hammond (1972) stated that apart from *F. hepatica* and *F. gigantica* there exists two other true fluke species. *Fasciola tragelaphi* reported from Southern Rhodesia (now Zimbabwe) and Uganda found parasitising Kudu and Bushbucks and from East and Southern Africa *Fasciola nyanzae* in Hippopotamus.

Opinions on fluke classification in the Americas differ. Sinitzin (1933) described *Fasciola halli* and *Fasciola californica* although they do not exibit many morphological deferences to the two common liver flukes *F. hepatica* and *F.*

gigantica. Price (1953) noted morphological variations in flukes collected from different parts of the United States of America with some close to *F. hepatica,* those from Texas and Florida identical with *F. gigantica* and most of the Gulf isolates were morphologically intermediate forms between *F. hepatica* and *F. gigantica.*

Malek (1980) summarised the taxonomy of liver fluke and concluded that although there are taxonomic disputes in various geographical areas there are only two most important valid species namely *F. hepatica* and *F. gigantica.*

2.1.3 Life Cycle of *F. hepatica* and *F. gigantica*

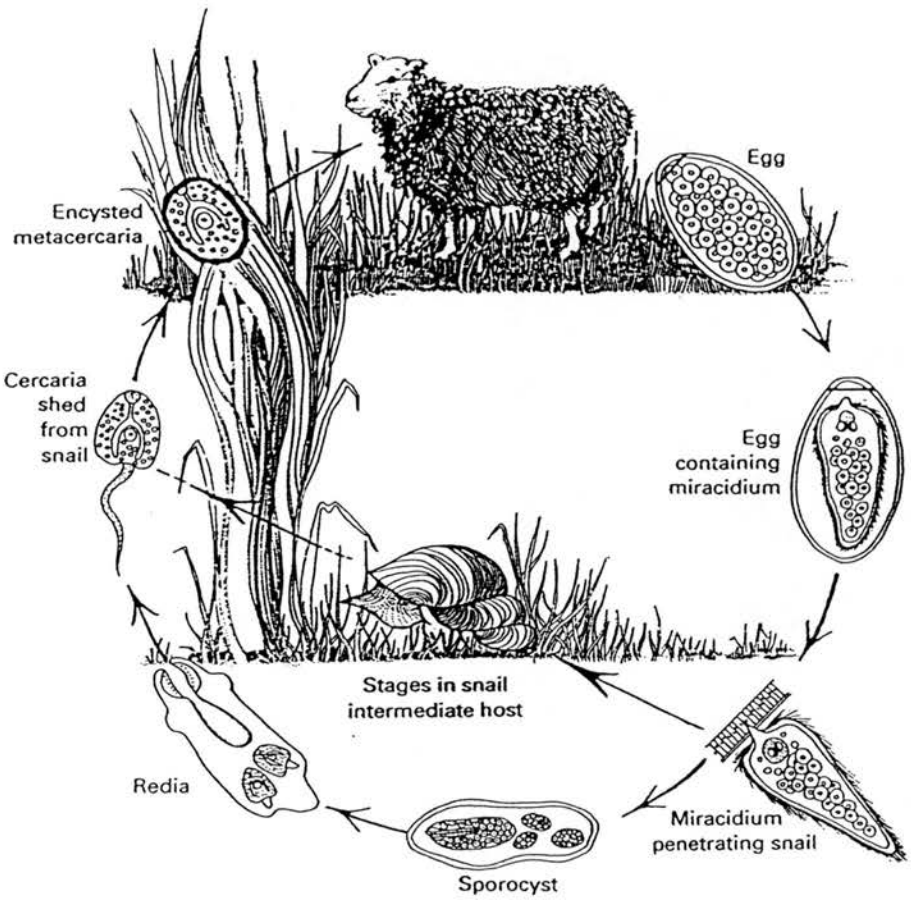


Figure 2.1: The life cycle of *Fasciola* spp. (after Urquhart, Armour, Duncan, Dunn and Jennings, 1996)

The life cycle of liver flukes was not known until early 1880s when two independent investigators, Leuckart and Thomas published the results of their work (quoted by Reinhard, 1957) .

Adult flukes lay their eggs in the bile ducts of the definitive hosts, typically sheep, goats, cattle and buffaloes, and the eggs enter the duodenum with the bile, together with the faeces leave the mammalian host and enter the environment (Georgi, 1980; Soulsby, 1982; Blood and Radostits, 1989; Bürger, 1992). Soulsby (1982) stated that the further development of the egg depends on the moisture content of the environment, importantly temperature and availability of the intermediate host. The general life cycle of *F. hepatica* and *F. gigantica* is shown in Figure 2.1. *F. hepatica* has a land based intermediate host and *F. gigantica* has an aquatic snail host.

Malek (1980) reported that it takes 10 to 15 days at a temperature of 23 to 25°C to hatch *F. hepatica* while Soulsby (1982) estimated 10 to 12 days at 26°C. Ogambo-Ongoma and Goodman (1973) and Dinnik and Dinnik (1959) showed that *F. gigantica* requires a minimum of 17 days and a maximum of 111 days to hatch at a constant temperature of 26^0C.

The process of hatching releases the miracidia which actively attack the soft foot of the snail and penetrate the snail tissue. A cycle of development, which is temperature dependent (Dinnik and Dinnik, 1964; Gorgi. and Theodorides, 1980; Soulsby, 1982), then follows with the parasite passing through sporocyst, rediae and

daughter rediae stages. This cycle takes from 45 days to about 100 days in the snail after which cercariae starts to release from the snail. Dawes (1960) reported that the miracidium adheres by suction to the epithelial cells of the snail and breaks these down probably by means of enzymes secreted by apical organs of the gut and cast off the ciliated epithelium as it enters the snail as a young sporocyst. Dawes (1960), Kendall (1965) and Malek (1980) considered that miracidia change to sporocysts near the site but before penetration, the young sporocysts then penetrate the snails. Roberts (1950), Soulsby (1982) and Bürger (1992) on the other hand believe that the sporocysts are formed after the miracidia have penetrated the snail.

F. hepatica sporocysts are usually found in the mantle edge, the kidney and oesophageal area of the snail where mother rediae from after about 2 to 3 weeks (Dawes, 1960; Malek, 1980; Soulsby, 1982). Most *F. gigantica* sporocysts are localised along the tissue surrounding the respiratory cavity, the pre-oesophageal region and the mantle of the snail (Dinnik and Dinnik, 1963; Kendall, 1965).

The rediae usually leave the sporocysts by rupturing the sporocyst wall and then migrate towards the digestive gland or hepato-pancreas, of the snail. It is in this digestive gland where, under favourable conditions, the rediae develop into tailed infective larvae or cercariae. In the case of *F. gigantica* however if the factors are not conducive daughter rediae develop before cercariae (Kendall, 1965).

Development from miracidium to cercariae in *F. hepatica* takes two months (under favourable conditions) according to Bürger (1992) or 56 to 86 day as reported by Malek (1980) and 36 to 40 days for *F. gigantica* (Dinnik and Dinnik, 1963).

The infective stage of *Fasciola* species, metacercariae are normally found as small white cysts on the grass or herbage on low lying damp ground. These white cysts encysts from the free swimming tailed larval stages, cercariae, shed by the intermediate host after actively reaching the herbage (Hughes, 1985; Georgi, 1985). *F. gigantica* also encyst after attaching themselves to any object in shallow waters especially floating leaves and plants (Cheruiyot and Wamae, 1990).

The definitive host becomes infected after ingestion of the plants harbouring metacercariae (Hughes, 1985; Losos 1986; Malek, 1980; Sewell and Hammond, 1974; Hammond and Sewell, 1990; Bürger, 1992). At the time Schumacher (1938) started his series of experiments there were three possible routes by which the fluke was thought to reach the bile duct in the liver of their definitive hosts and these were:

(a) Via the duodenum and against the bile stream into the bile ducts

(b) Via the blood stream by way of portal vein and

(c) Through the abdominal cavity and the liver parenchyma to the bile ducts .

Schumacher (1938) concluded that the fluke's route of invasion in a definitive host is through the abdominal cavity. He then carried out the same experiments in sheep where he demonstrated the presence of young flukes in the abdominal cavity, the liver parenchyma and after only eight weeks in the bile duct (Schumacher, 1938).

It is now an established fact that the migration of *F. hepatica* and *F. gigantica* in the mammalian host starts with penetration of the duodenum by the young fluke or Marita (Georgi 1985). This marita passes though the peritoneal cavity until it finds the liver capsule. After crossing the capsule the young fluke passes through the liver

parenchyma causing tissue damage before entering the bile ducts. The maturation of the marita to an adult fluke takes place in the bile ducts (Reinhard, 1957; Boray, 1969; Malek, 1980; Georgi, 1985; Bürger, 1992) .

From infection to a mature fluke, *F. hepatica*, in the bile ducts takes a minimum period of 8 weeks (Schumancher, 1938; Bürger, 1992) and at least 11 weeks for *F. gigantica* (Hammond, 1974).

2.1.4 Morphology of the Different Life Cycle Stages

The adult fluke (*F. hepatica* and *F. gigantica*)

The two major economically important *Fasciola* spp, *F. hepatica* and *F. gigantica*, like most members of the *Phylum* Platyhelminthes are soft boded, flatted dorsoventrally and hermaphroditic (Georgi, 1985; Bürger, 1992). Jeffrey and Leach (1966), Malek (1980) and Soulsby (1982) described the shape of these two flukes as leaflike. Both species have two suckers, one surrounding the mouth and a slightly larger suckers on the ventral side, close to each other in a cone-like anterior extension of the body. The posterior end of the fluke is usually pointed.

Fluke internal organs are branched (Jeffrey and Leach, 1966; Malek, 1980). The intestinal caecae reach to the posterior end, numerous branches of the testes fill the middle half, and the single ovary is situated on the right side in front of the anterior branches of testes. Also vitellaria are dorsal and ventral to the caecae covering the whole lateral hindbody of the fluke (Malek, 1980; Soulsby, 1982; Jones and Hunt, 1983). Soulsby (1982) clearly showed that *F. gigantica* though similar to other *Fasciola spp*. differs in that it has more branched *caecae*, has a longer and more

numerously branched ovary while that of *F. gigantica* has fewer club shaped branches.

Malek (1980) gave a range from 20 to 50 mm in length and 6 to 12 mm in width for *F. hepatica* and from 30 to 70 mm by 3 to 11 mm for *F. gigantica*. Soulsby (1982) and Bürger (1992) suggested that *F. hepatica* may reach 30 by 13 mm and that of *F. gigantica* 70 by 12 mm.

Sahba, Arfaa, Farahmandian and Jalali (1972) examined the average length to width ratios of the two species and concluded that for *F. gigantica* the ratio is 4.39-5.20:1 and for *F. hepatica* 1.88-2.32:1. The size of adult flukes depends on several factors, including the age of these parasites, the species of their definitive host and the intensity of the infection (Kendall, 1965; Boray, 1969; Hammond, 1972).

The Ovum

Egg morphology has long been used to differentiate individual fluke species (Taylor, 1964; Jeffrey and Leach, 1866; Malek 1980; Urquhart, Armour, Duncan, Dunn and Jennings, 1987; Bürger, 1992). Ogambo-Ongoma and Goodman (1973) and Malek (1980) described *Fasciola spp.* eggs as yellowish brown, ovoid, unembryonated, operculated with a clear nuclear area near the opercular. The eggs contents was described as dark aggregations with uneven masses surrounding the clear nuclear area. *F. gigantica* ova are larger than that of its temperate sister species, *F. hepatica.*

Kendall and Parfitt (1959) and Jeffrey and Leak (1966) stated that *F. hepatica* eggs are 130 to 150 microns long and 63 to 90 microns wide while *F. gigantica* eggs

160 to 190 microns long 70 to 90 microns wide. Malek (1980) reported that the average size of *F. gigantica* eggs collected from the Sudanese cattle to be 152 by 92 microns and 136 by 80 microns for eggs from cattle from Louisiana USA.

Soulsby (1982) noted the length of eggs as 130 to 150 microns and the width range of 63 to 90 microns where as *F. gigantica* eggs measurements range from 156 to 197 microns for length and 90 to 104 microns for width. Care needs to be taken if the size and shape of these eggs are measured after storage because according to Hammond (1972) eggs may change in shape and size after storage.

The Miracidium

Troncy and Vasseau-Martin (1974) defined the miracidium of *F. hepatica* as a mobile larva characterised by a ciliated locomotion system, an anterior papilla and cephalic glands permitting the penetration of the host's tissue, two paired eye spots, two excretory vibratile flame cells and germinal cells. Ogambo-Ongoma and Goodman (1973) observed that the larvae of *F. hepatica* which hatch from fluke eggs are tiny, densely ciliated organisms and measure 156 by 58 microns. Malek (1980) gave the size of miracidium as being 130 by 28 micron and that it has a ciliated epithelium with 21 polygonal cells arranged in five rings of six, six, three, four and two cell. He also pointed out that under the epithelial layer is syncytium with two layers of muscle fibres, circular and longitudinal and beneath these muscle layers are large vesiculated cells of about 10 microns in diameter. Little is known about *F. gigantica* it is assumed however that it is similar to *F. hepatica*.

The Sporocyst

The next stage of the development of flukes in the snail host is a sporocyst which is an example of "regressive metamorphosis" and is distinguished by the absence of a locomotion system, the variation of its shape, the regression of the internal composition present in the miracidium and the presence of genital cells (Troncy and Vasseau-Martin 1974). The sporocysts first appear as an elliptical shaped sack measuring 0.15 mm they then grow to 0.5 mm after fifteen days. Three weeks after initial infection of the snail the sporocyst start to produce rediae (Malek, 1980).

The Rediae

The rediae are characterised by (a) the presence of a locomotion system and a structure made up of anterior, muscular collar and two posterior appendices which become turgescent when the collar contracts (b) a sack-like, functional alimentary tube (oesophagus) with a typical, muscular pharynx, an excretory system consisting of two vibratile flame cells (selected location within the hepatopancreas and large number of germinal cells) which develop into new forms of rediae and cercariae (Dinnik and Dinnik, 1959; Troncy and Vasseau-Martin, 1974). Malek (1980) suggested that the rediae when fully grown can measure as much as 0.78 mm taking a cylindrical shape with a raised collar near the anterior end and two projections in the posterior third of the body.

According to Ollerenshaw and Graham (1986) one can differentiate the rediae of *F. hepatica* from that of *F. gigantica* simply by comparing their blind intestines.

The blind intestines of *F. hepatica* rediae are short and globose commonly one sixth but can be up to a third of the length of the body while these of *F. gigantica* always occupy more than a third and up to half of the body length.

The Cercariae

The cercariae develop from rediae and have a large almost round spone shaped body of 280 to 320 microns long by 250 microns wide, with a long motile tail measuring approximately 700 microns long (Malek, 1980). The cercariae have four types of cytogenic cells, characterised by their position and histochemical properties (Dixon, 1966). Georgi (1985) summarised the morphology of cercariae as a tadpole-like larvae with a discoidal body and a long tail for swimming. This larval stage displays certain adult organs including oral and ventral suckers, mouth, pharynx, forked intestines, excretory canals with flame cells and a premordia of the reproductive organs.

The Metacercariae

After the cercariae attach to the herbage and encyst they form the larval stage usually referred to as metacercariae, the infective form of *Fasciola* for its mammalian definitive host. This stage is non-sesile and it is surrounded by four layers of protective cyst walls. It is within this protective cyst that the development takes place. This development includes the continued formation of premordial reproductive structures. The cyst containing a motile *Fasciola* embryo measures approximately 0.2-0.25 mm in diameter (Malek, 1980; Ershov, 1960). This embryonic stage has very well-defined suckers, a branch of gut and an excretory bladder.

2.1.5 Production of Metacercariae in the Laboratory

All work with experimental infection of animals with *Fasciola spp* depends upon the supply of viable metacercariae. The two most important intermediate host groups are amphibic snails represented by *Lymnaea* spp and include *Lymnaea truncatula* and aquatic group of *Lymnaea* spp. which including *Lymnaea auricularia* (Kendall and Parfitt, 1965; Boray, 1969; Malek, 1980).

Laboratory maintenance and management of snails

Taylor and Mozley (1948), first developed ways for culturing *L. truncatula* consisting essentially of simulating the natural habitat of the snail in unglazed earthen- ware pans or shallow glass dishes. Each of these containers had a small pool of water and a mud slope on which green algae (*Oscillatoria brevis*), the principle food of the snail, grew. Kendall (1953) reported the production of many uniform colonies of *L. truncatula* using this method.

Snails are kept at a temperature a little above 20°C, these temperatures are usually controlled under laboratory conditions (Kendall and Parfitt, 1965). Kendall (1953) reported that *L. truncatula* remain active at a temperature as low as 1.5°C and that the optimum temperature for reproduction and growth ranges from 18° to 21°C, mainly because at this range a vigorous growth of green algae is sustained. He, however, warned that a constant temperature above 20°C may be regarded as unfavourable while even occasional temperature above 25°C should be avoided. Whitlock, Campbell, Chow, Rolfe, Porter and Kelly (1977) however managed to

maintain *Lymnaea tomentosa* at a constant temperature above 20°C (approximately 23°C).

Kendall (1964) reported that when infected with *F. hepatica, L. truncatula* to be maintained between 10^0C and above 28°C. When snails are infected, the temperature is to be taken into consideration and should be a balance between that which suits development of the snails, of the *Fasciola* in the snails and the growth of green algae in case of *L. truncatula*. Mahato (1993) maintained a constant temperature of forced aerated aquaria at 25°C. *L. natalensis* and *Lymnaea auricularia* kept in forced aerated aquaria have tolerated slightly higher temperatures than their amphibic counterparts (Hammond, 1970; Mahato, 1993; Madsen and Monrad, 1981).

The intermediate host of *F. hepatica* grow and thrive well with green algae culture as feed and should be changed to fresh algae regularly (Kendall, 1953; Lee, Kim and Lee, 1994; Urquhart Personal Communication, 1994). Kendall and Parfitt (1965) mentioned the use of an artificial food i.e. modified calcium alginated gel. *L. natalensis* can be fed on aquatic algae but can also be fed on lettuce and Tetramin® (fish food) (Madsen and Monrad, 1981). Hammond (1970) kept *L. natalensis* in aerated aquaria with aquatic plants which increased the surface area for algal growth for the snails to feed on. Mahato (1993) maintained *L. auricularia race referscens* collected from the field in glass sealed aquaria under fluorescent light to help the algal growth. He also supplemented with fish food flakes (tropical fish food 'Vetra Betta' Singapore) every other day.

Collection and preparation of *Fasciola spp.* eggs for culture

Fasciola spp. eggs used for producing metacercariae can be collected from the gall bladder after infected animals have been slaughtered (Schumacher, 1938; Dinnik and Dinnik, 1959; Boray, 1969; Mahato, 1993). However eggs can also be collected from the faeces of experimentally infected animals, as long as their egg counts are high enough (Anon, 1977; Madsen and Monrad, 1981). Collecting eggs from the bile involves washing the eggs, usually by repeated sedimentation in cold water and carefully discarding the supernatant (Dinnik and Dinnik, 1959; Kendall, 1966; Sindou, Cabaret and Rondelaud, 1991). Faecal egg collection demands removal of the debris by use of a sieve with a large enough mesh to let through the eggs but retain most of the debris. This is followed by washing as described previously (Madsen and Monrad, 1981).

The final wash removes further debris by use of multiple sieves with a large meshed sieve first ending with small meshed sieve to trap the eggs. The number of sieves used in this process varies from one (Boray, 1966) two (Mahato, 1993) to three (Sindou, Cabaret and Rondelaud, 1991). Sindou, Cabaret and Rondelaud (1991) sieved the eggs with gradual decreasing mesh sizes from 75 microns, 50 microns and 38 microns. The eggs trapped in the last sieve are then washed into a beaker ready for incubation. Mahato (1993) used just two sieves one to trap debris, 150 mm mesh size, and 38 mm to trap the eggs. Although time consuming, Dinnik and Dinnik (1959) and Ogambo-Ongoma (1971) washed the eggs by repeated sedimentation in cold clean water.

Culturing miracidia from fluke eggs

After cleaning the *Fasciola* spp. eggs are divided in small enough aliquots to form a monolayer in an appropriate container for incubation (Kendall, 1966). Kendall (1966), Roberts (1950) and Boray (1969) used Petri dishes. Dinnik and Dinnik (1959) used glass beakers and Ajanusi (1993) and Urquhart (1994) personal communication incubated the fluke eggs in amber coloured glass bottles. The eggs are incubated in clean water either straight from the tap or any other clean water source (Dinnik and Dinnik, 1959) or deionised water (Kendall, 1966; Boray, 1969).

The most important factor is the temperature under which the eggs are incubated. Ollerenshaw (1959) and Rowcliffe and Ollerenshaw (1960) showed that 9.5-10°C is the minimum critical temperature for both eggs of *F. hepatica* and *F. gigantica* to hatch viable miracidia. It took Boray (1969) 90 to 100 days to hatch *F. gigantica* eggs maintained at 11°C but only 21 days at an average temperature of 15°C. Dinnik and Dinnik (1959) failed to notice any hatched eggs in winter when the minimum water temperature was 5.5°C and 19.5°C as a maximum. Roberts (1950) in his effort to examine factors affecting development and hatchability of *Fasciola* spp. eggs found he was able to hatch the eggs in about 14 days at 25°C. Incubation in the laboratory takes place in a lightproof environment (Kendall, 1966; Boray, 1969; Ajanusi, 1994; Mahato, 1993). Light is important as a stimulating factor at the time of hatching. When the miracidia are developed the eggs are placed under a strong light and within minutes the eggs hatch (Roberts, 1950; Kendall, 1966; Boray, 1969; Ajanusi, 1994). Roberts (1950) reported that blue and violet wavelengths do not induce hatching. The duration of incubation depends on many

factors i.e. temperature, moisture and *Fasciola* species being the most important. It takes longer for *F. gigantica* eggs to hatch than *F. hepatica* (Kendall, 1967).

Infecting snails with miracidia

Boray (1969) infected 4 to 6 weeks old *L. tomentosa* (4-6 mm) and placed them in groups of ten in plastic dishes with deionised water. Instead of infecting the snails with already hatched miracidia he put 20 eggs containing fully developed miracidia for each snail in each group. Anon (1977) recommended that *L truncatula* are infected when their shells are 2-3 mm long i.e. 2-3 week old. Ajanusi (1994) infected *L. truncatula* when the shells were 2 - 5 mm long using flat-bottomed polystyrene microwell plates (Gibco Ltd, (code 2-62162A). Sindou, Cabaret and Rondelaud (1991) and Whitlock, Campbell, Chow, Rolfe, Porter and Kelly (1977) infected in groups of 25 snails in Petri dishes.

Three to six hours contact is required for the infection to take place (Ajanusi, 1994; Sindou, Cabaret and Rondelaud, 1991; Mahato, 1993). It is important that the water in which the infection takes place and temperature corresponds to that which snails tolerate i.e. for *L. natalensis* aquarium water and for amphibic snails deionised water (Boray, 1969; Sindou, Cabaret and Rondelaud, 1991).

Shedding of cercariae by snails

Schumacher (1938) realised that keeping snails harbouring mature (6 weeks or more post infection) *F. hepatica* rediae at 10°C then bringing the temperature up to room level induces shedding of rediae by the snails. The use of the 'Cold shock' method is the current method of choice of many researchers (Ajanusi, 1994; Mahato,

1993; Kendall, 1964). In contrast Taylor (1964) reported that direct sunlight or 60 watt light bulb stimulates cercariae shedding. Kendall and McCullough (1951) and Kendall (1965) were of the opinion however that light doesn't stimulate shedding of cercariae by the snail.

According to Standen (1963) however, this cold shock method does not work as well for *L. natalensis* (*F. gigantica*) as it does for *L. truncatula* (*F. hepatica*). He also found that the cercariae shedding is more frequent at night than during then day for *F. gigantica*. Hammond (1970) reported an increased shedding of *F. gigantica* cercariae shortly after removing the snails from a light to a dark environment. Moving snails to fresh water also induces shedding, Boray (1969) reported that flushing stagnant snail habitats and particularly watering the snail habitat induced massive shedding of cercariae. After the snails shed the cercariae (4-5 hours) they are returned to the aquarium or *algae* tray. The cercariae are left to stand at room temperature overnight (Anon, 1977) or 16 hours (Boray, 1969) in order for the metacercariae to form.

The container into which the shedding takes place should be convenient for collection of the metacercariae especially to avoid physical damage (Urquhart Personal Communication, 1994).

2.2 EPIZOOTIOLOGY

2.2.1 Geographical Distribution of *F. hepatica* and *F. gigantica*

The two species, *F. hepatica* and *F. gigantica*, have world-wide distribution with the former species having a wider range than it's tropical relative (Malek, 1980; Losos, 1986; Over, 1982).

Fasciolosis caused by *F. gigantica* is widespread throughout Africa, Asia and the Pacific (Losos, 1986; Malek, 1980; Soulsby, 1982). According to Malek (1980) the flukes that Prince (1952) described in Southern United States of America (USA) may belong to the species *F. gigantica*. Blood and Radostits (1989) reported that *F. gigantica* is also found in the Southern USA. *F. hepatica* on the other is found in North America, Europe, Northern Asia, Northern Africa and Australia. It has also been reported in the highlands of Kenya, South Africa and in Central and South America and on many of islands including Japan, New Zealand, Cyprus, New Guinea and Iceland (Malek, 1980; Over, 1982)

In some parts of the world, the geographical distribution of *F. hepatica* overlaps that of *F. gigantica.* Kendall (1954) found *F. gigantica* mostly in the lowlands and *F. hepatica* in the highlands of Pakistan but recorded mixed infection in an intermediate zone. Although Magzout and Kasim (1978) reported the presence of the two *Fasciola spp.* in animals slaughtered at abattoirs in Saudi Arabia, it is clear that animals were imported into the country and that the species found were in line with the country of origin i.e., the Sudan and Eastern Europe.

Since the incidence of fasciolosis is often measured simply by abattoir liver condemnations, the countries with a developed abattoir infrastructure give more accurate figures than those with poor infrastructure. However, prevalence differs from region to region within the same country due to numerous factors for example agroecological zones and intermediate host habitation (Over, 1982).

Table.2.1: The prevalence of fasciolosis (*F. hepatica* and *F. gigantica*) as it stands in some of the most recent reports in some countries in the World. The majority countries are on the African continent.

Country	Species	Prevalence	References
Cameroon	*F. gigantica*	45	Malek (1980)
Ethiopia	*F. gigantica*	30-90	Malek (1980)
Kenya	*F. gigantica*	19.8	Cheruiyot (1987)
Mozambique	*F. gigantica*	70.0	Alves (1970)
Nigeria	*F. gigantica*	65.0	Schillhorn Van Veen (1980).,
Nepal	*F. gigantica*	18.5	Mahato (1993)
Pakistan	*F. gigantica*	70.0	Kendall (1954)
Tanzania	*F. gigantica*	41-50	Hammond (1965)
Zambia	*F. gigantica*	89.6	Silangwa 1973)
Zimbabwe	*F. gigantica*	50.0	Chambers (1987)
The Republic of Ireland	*F. hepatica*	38.0	Dargie (1989)
The UK	*F. hepatica*	6.0	Dargie (1989)
The USA	*F. hepatica*	4.4	Malone *et al.(*1982).

2.2.2 Transmission of Fasciolosis

Transmission depends on several factors related to the biology of the vector, the biology of the parasite and stock management (Troncy, 1989). In many tropical and subtropical regions, feed and water scarcity forces herdsmen to move long distances with their livestock. Inevitably this leads to crowding at waterholes or grazing areas. This in turn exposes the animals to high levels of infestation by metacercariae (Hammond and Sewell, 1990; Losos, 1986). In Australia, where the conditions are dry and hot, infections are usually due to recent contamination of the environment (Meek and Morris, 1979), while in temperate region such as the United

Kingdom and most of Europe, massive levels of metacercariae can accumulate over a period of time (Ross, Dow and Todd, 1967).

The transmission of *Fasciola spp.* is complicated by involvement of two species of *Lymnaea* one amphibic *Lymnaea* i.e. *L. truncatula* with world-wide distribution (Urquhart, Armour, Duncan, Dunn and Jennings 1987) and the other aquatic found in tropical countries Hammond and Sewell (1990) and Losos (1986). Although the two species appear to be specific in their intermediate hosts, Boray (1969) was able to show that *L. tomentosa* is a capable vector for *F. gigantica.*

Table 2.2 Some of snail subspecies responsible for the transmission of *Fasciola spp.* in respective countries or regions

F. hepatica		
Snail species	**Country (ies)**	**References**
Lymnaea truncatula	Europe and West Asia	Kendall, (1965)
Lymnaea. mweruensis	Kenya	Dinnik and Dinnik (1963)
Lymnaea. humilis	Canada and part of USA	Malek (1980)
Lymnaea. viatrix	South America	Malek (1980)
Lymnaea tomentosa	Australia	Boray (1969)
Fossaria modicella	North America	Malek (1980)
Fossaria. bulimoides	Gulf of Mexico States	Malek (1980)
F. gigantica		
Fossaria. auricularia	India and Pakistan	Mahato (1993)
Lymnaea natalensis	Most of Africa	Bürger (1992)
Lymnaea rufescens	Pakistan	Kendall (1965)
Lymnaea rubiginosa	Malaysia	Hammond and Sewell (1990)
Lymnaea gedrosiana	Iran	Malek (1980)

2.3 PATHOGENESIS OF FASCIOLOSIS

2.3.1 Clinical Signs

Ross and Todd (1968) reported that the severity of the clinical symptoms is approximately related to the level of fluke infestation in the liver, however they also reported evidence of resistance in some animals. According to Schillhorn van Veen,

(1980), Blood and Radostits (1989), Malek (1980), Losos (1986) and Urquhart, Armour, Duncan, Dunn and Jennings (1987), three partially overlapping clinical syndromes are observed in cattle and small ruminants and include:

- The acute disease caused by immature worms migrating through the liver parenchyma;
- The subacute form, characterised by anaemia and caused by the young adult flukes emerging from the liver parenchyma into the bile ducts; and
- The chronic form which is a wasting disease with presence of flukes in the bile ducts.

Acute syndrome

Acute fasciolosis occurs during invasion of the liver by recently ingested metacercariae (Georgie, 1985; Bürger, 1992; Malek, 1980; Soulsby, 1982; Losos, 1986). Soulsby (1982) further reported that in acute cases, especially in sheep, the animal dies suddenly with blood stained foamy exudate appearing on the nostrils and blood is discharged from the anus (as in the case of anthrax). When observed, the clinical signs in sheep consist of apathy, lack of appetite, pallor and oedema of mucosae and conjunctivae, and pain when pressure is exerted over the area of the liver (Blood and Radostits, 1989). Losos (1986) reported in addition an occasional appearance of jaundice. Outbreaks are very rare but may appear in young animals. A complication of the acute condition is black disease caused by anaerobic bacteria (*clostridium novyi* type B) which proliferates in the necrotic lesions produced by immature flukes (Soulsby, 1982; Hammond and Sewell, 1990).

Acute fasciolosis is common in small ruminants, goats and sheep and usually results in death without other apparent clinical symptoms (Ross, Dow and Todd, 1967). Hammond and Sewell (1990) also suggested that acute fasciolosis is common in small ruminants especially if infected with *F. gigantica*. Soulsby (1982) however, pointed out that, in the field, acute fasciolosis is less common than the chronic fasciolosis in both small and large ruminants. Schillhorn van Veen (1980) pointed out that although acute fasciolosis is a rare form of the disease in cattle but it causes great damage when it does occur.

Subacute syndrome

The subacute syndrome has a longer course than acute fasciolosis and is usually associated with the emergence of young flukes in the bile ducts and ingestion of large amounts of metacercariae over a long period (Blood, 1994; Urquhart, Armour, Duncan, Dunn and Jennings, 1996). The prominent clinical symptoms are pallor of the mucosae and some oedema in the submandibular area. Pain on pressure over the liver is a common observation (Blood, 1994; Bürger, 1992). Boray, (1969) mentioned that in subacute fasciolosis sheep do not die earlier than seven to eight weeks in agreement with Fraser (1991) the survival duration in subacute fasciolosis is 7 to 10 weeks. Death does occur due to haemorrhages and anaemia.

Chronic syndrome

Chronic fasciolosis occurs when small numbers of metacercariae have been ingested over a long period (Blood and Radostits, 1989). According to Soulsby (1982) the syndrome is the most common form of fasciolosis in sheep, cattle and

other hosts including man. In tropical countries this syndrome is mainly observed at the end of the dry season (Schillhorn van Veen, 1980) The main symptoms in sheep are loss of body weight, pallor of the mucosae over a period of weeks and submandibular oedema (Blood and Radostits, 1989). Losos (1986) reported that in *F. gigantica* infection subcutaneous oedema may also develop on the face and lower regions of the abdomen and thorax. Dairy cattle also lose weight and suffer a reduction in milk production (Blood, 1994). Eckert (1992) reported chronic diarrhoea due to fasciolosis, but Urquhart, Armour, Duncan, Dunn and Jennings (1996) emphasised that diarrhoea is not a clinical feature of bovine fasciolosis unless complicated by *Ostertagia spp* infection. Development of acute clinical disease has been observed in animals with chronic fasciolosis in under nourished animals especially at the end of dry season (Graber, 1979).

2.3.3 Clinical Pathology

Anaemia

Anaemia is perhaps the most mentioned symptom of subacute and chronic fasciolosis. The results obtained after analysing blood samples from animals suffering from either *F. hepatica* or *F. gigantica* infection for 10 weeks or over always show a decrease in red blood cells, packed cell volume and haemoglobin concentration (Blood, 1994). There have been reports (Ogunrinade, 1984) of terminal macrocytic anaemia in animals which died of acute fasciolosis. Sinclair (1964) found a totally different type of anaemia, macrocytic hypochromic anaemia or classical iron deficiency, seen as a late result of blood loss. This is in agreement with

Ross, Dow and Todd (1967) who reported the macrocyotosis with erythroblastosis in *F. hepatica* infection. Although Bürger (1992) observed a macrocytotic and hypocromic anaemia in fasciolosis, the majority of authors are of the opinion that *Fasciola spp.* infection causes a normocytic and normochromic anaemia (Boray, 1967; Roberts 1968; Bürger, 1992; Blood, 1994). As in the clinical syndrome the severity of anaemia depends on the fluke burden, the nutritional status of the host and the stage, as well as the duration, of the infection. Anaemia is not only the most common clinical symptom of subacute and chronic fasciolosis but probably the most characteristic of these two syndromes (Sewell, 1967).

Boray (1967) reported that all sheep infected with 200 to 700 *Fasciola hepatica* metacercariae showed chronic progressive anaemia from about 12 weeks after infection. Severe anaemia developed from about the fifth week after infection in all sheep harbouring approximately 100 or more flukes. Kadhim (1976) working with *F. gigantica* also recorded a progressive anaemia beginning 12 weeks post infection, with the lowest values occurring in the 14 weeks post infection, in all except for one sheep infected with 200 metacercariae.

Ogunrinade (1984b) compared *F. gigantica* infection in sheep and goats and reported that anaemia was pronounced in both species of animal, in the eighth week post infection with onset of anaemia as early as in the sixth week. Roberts (1968) found that the onset anaemia in acute *F. hepatica* fasciolosis in sheep occurred as early as three weeks after infection in groups of sheep, one receiving single infection

of 5000 metacercaria and the other multiple infection of 1000 metacercariae given every third day for five days. The severe anaemia lasted for 6 weeks post infection.

Hammond (1970) reported a reduction in packed cell volume, haemoglobin concentration and red blood cell counts in sheep 2-4 weeks after infection with *F. hepatica* metacercariae. In contrast Sewell (1966) experimentally infected a Zebu steer with 2000 metacercariae of *F. gigantica* and observed anaemia about 8 weeks later.

Conflicting ideas have been forwarded by researchers regarding the cause of anaemia in fasciolosis, Jennings, Mulligan and Urquhart (1956) were able to quantitatively measure the blood loss using ^{32}P labelled red blood cells and recorded a 0.2 ml blood loss per fluke per day. This was an underestimation according to Holmes, Dargie, MacLean and Mulligan (1968a) who used ^{51}Cr and ^{59}Fe labelled cells and found the red blood cell loss to be nearly 0.5 ml per fluke per day. This phenomena, of blood loss due to haemorrhage caused by migrating Flukes and fluke feedingon blood is supported by many other reports (Todd and Ross, 1966; Hammond, 1970; Boray, 1967, 1969 and Bürger, 1992). Contrary to this aetiogical hypothesis, Dawes and Hughes (1964) reported evidence that flukes fed on hyperplastic epithelium in the bile duct of a mouse. They suggested therefore that anaemia may not be due to blood sucking. Sinclair (1965) supported this conclusion and stated that sheep he bled daily had a higher rate of iron utilisation by erythrocytes than *F. hepatica* infected sheep. He suggested therefore that the anaemia was due to a secondary disorder of the reticulo-endothelial system. On the other hand Symons

and Boray (1967) were able to show that when intact flukes are removed from their attachment sites the bile duct mucosa was missing and the region was occupied by a blood clot containing erythrocytes. Symons and Boray (1967) further argued that the results of their experiments showed clearly that the transfer of ^{59}Fe to the bone marrow and from thence to erythrocytes was so rapid that the rate of erythropoiesis was greatly increased.

Cameron (1951); Ershov (1960) and Lapage (1968) suggested involvement of pathologic substances in the pathogenesis of fasciolosis. Isseroff, Spengler and Charmock (1979) working with rats suggested that proline as a pathologic product could also be involved in the aetiology of anaemia. When Ogunrinade and Makinde (1988) tried to examine the effect of proline in rabbits they did not record any significant effect in either erythrocytes or ^{59}Fe clearance and utilisation. The role of Proline (an amino acid) has been examined by Isseroff, Sawina and Re Reino (1977) as a contributing factoir in the causes of hyperplasia of the bile duct of the *Fasciola spp.* infected animals.

Boray (1967) and Sinclair (1967) suggested that the deficiency of vitamin B_{12} might not be the cause of anaemia after injecting anaemic sheep with vitamin B_{12} and found that there was no improvement.

Eosinophilia

Eosinophilia, localised and systematic, is a common feature in helminth infection of domestic animals and this includes *Fasciola* spp. infection (Malek, 1980). Eosinophilia appears soon after infection, increases to a high level while the

flukes are migrating, and subsides when the flukes have reached the bile ducts (Malek 1980). Kadhim (1976) reported a sharp increase in eosinophil counts which increased to 17.5% of all white blood cell counts in the second week of *F. gigantica* infection in sheep. After 4 weeks the counts reached the peak (20%). Thereafter, it fluctuated but remained consistently higher than that of the control group

Changes in serum total protein and albumin levels

The liver is the only source of albumin and damage will cause hypoproteinaemia and hypoalbuminaemia. The hypoproteinaemia and hypoalbuminaemia have been reported in *F. gigantica* infections of different hosts (El-Samani, Mahmoud, Fawi, Gameel and Haroun, 1985; Wiedosari and Copeman, 1990; Mahato, 1993; Wamae, 1996) and in *F. hepatica* infection of different hosts (Boray, 1969; Urquhart, Armour, Duncan, Dunn and Jennings, 1996).

Serum total protein is almost always considered in the context of general pathogensis or when anaemia is being examined as well as the effects of the disease on the nutritional status of the animal (Roberts, 1968; Sewell, 1966; Bürger, 1992). Early in infection, during fluke paraenchymal migration, hyperproteinaemia, hyperglobulinaemia and hypoalbuminaemia occur (Bürger, 1992). The hyperproteinemia has been attributed to mobilisation of body protein to maintain labile protein levels, needed to replace blood cells, as well as other protein losses caused by the infection as a result of leakage of blood constituents into bile caused by the blood sucking activities of the liver flukes as well as direct leakage through the bile duct epithelium (Soulsby, 1982). Wamae, (1996) reported total serum proteins

remained low from 0-14 wpi. in both the uninfected and infected Friesians and Boran cattle, thereafter the serum proteins rose between 14 - 17 wpi. after which no differences were found between the infected and control groups of cattle. Thus the infected Friesians showed a 2% increase over their uninfected controls whilst the infected Borans had a 3% increase over their uninfected controls. There were no significant differences in the total serum proteins between both the control groups. Kadhim (1976) and Wamae, (1996) reported significant changes in serum protein levels in sheep infected with *F. gigantica* occurring at the stage when other blood parameters also changed.

Roberts (1968) reported, a progressive rise in gamma-globulin from the first week up to the third week post infection in *F. hepatica* infection in sheep and a fall in albumin levels resulting in a drop in the albumin/gamma-globulin ratio. After treatment of these infected sheep a temporary rise of gamma-globulin occurred accompanied by somewhat slower but continuous recovery of albumin. The hypoalbuminaemia is associated with general expansion in plasma volume and reduction in albumin synthesis following severe liver damage by migrating flukes (Berry and Dargie 1978; Bürger, 1992). Synthesis of albumin depends on availability of dietary protein which is affected in turn by feed intake. Increased albumin synthesis apparently diverts amino acids from anabolistic activity including building muscle and milk production with consequential lowered productivity (Berry and Dargie, 1978). According to the above authors the infected Borans were able to maintain superior albumin levels despite suffering more severe liver damage but did

lose more weight. Sheep, that catabolised the most albumin, also synthesised the most and survived longest.

Changes in serum enzyme concentrations

Liver function enzymes glutamate dehydrogenase (GLDH) and gamma-glutamyl transferase (γ–GT) are found in high concentrations in the mitochondria of hepatocytes and bile duct epithelium respectively (Doxey, 1983). Any damage to the hepatocytes causes release of the enzyme from cell mitochondria into the blood stream and are thus ideal for use in following the pathogenesis of fasciolosis.

The earlier rise of GLDH activity coincided with the migration of juvenile liver flukes in the liver parenchyma. This results in damage to the hepatocytes causing release of the enzyme from cell mitochondria into the blood stream. Thus later rise of γ–GT activity signals entry of the liver flukes into the bile ducts and trauma of bile duct epithelium due to their feeding activities. It has been reported that the intensity of these changes is indicative of intensity of infection and host susceptibility (Reid, Armour, Urquhart and Jennings, 1970), a fact that may be of use in serological and epidemiological studies.

The advantages in measuring plasma enzymes in ruminant fasciolosis are that the onset and the course of the disease can be demonstrated and the effect of therapy monitored (Hughes, Treacher and Harness, 1973). Thorpe and Ford (1969), after studying the changes in plasma activity of the enzyme glutamate dehydrogenase (GLDH), sorbitol dehydrogenase (SDH) and glutamic oxaloacetic transaminase (GOT) in sheep with both single and multiple infection, found that as flukes migrate

through the liver parenchyma SDH and GLDH increase, indicating parenchymal cell damage. GOT activities in the plasma were found to increase earlier than the other two enzymes and remained elevated for a long time. Simesen, Nielsen and Nansen (1973) compared the activities of GOT and γ–GT and concluded that the γ–GT estimation may be a more useful parameter than GOT for the evaluation of the hepato-biliary damage caused by fasciolosis in cattle. Sewell (1967) reported that GLDH was the best indicator of hepatic disturbance in ruminants infected with *F. hepatica* and further suggested that because GLDH was less affected with haemolysis than GOT, would find wider use in the diagnosis of fasciolosis.

Sykes, Coop and Robinson (1980) compared the activities of serum GLDH, γ–GT and aspartate amino-transferase (AST) and suggested that GLDH and γ–GT activities are more sensitive indicators of liver cell damage in chronic subclinical ovine fasciolosis than AST activities and that γ–GT may be more suitable as a diagnostic aid due to its greater stability. This view is shared by Rowlands and Clampitt (1979) who further showed that GLDH plasma activity indicates passage of immature flukes through the parenchyma and γ–GT indicate their migration into the bile ducts.

For the above reasons liver enzymes, GLDH and γ-GT, are a good method for the detection of early or late fasciolosis respectively in experimentally infected animals. Ferre, Barrio, Gonzalez-Gallego and Rojo-Vazquez (1994) reported an increase in serum GLDH activity in sheep after forty days of *F. hepatica* infection, the next was AST 80 days and although serum γ-GT activities increased as early 80

days it took 120 days to peak in some sheep. Wamae, (1996) reported that in infected Friesian cattle, serum GLDH activities first increase 4 wpi., while in infected Boran cattle, enzyme activities increased in the 4 wpi. Peak activities of the enzyme occurred at 13-14 wpi. in the infected Friesians and Borans respectively. The activity of serum γ-GT increased from the twelfth week of infection in both infected groups reaching peak activities at 16 wpi. between 13 and 16 wpi. the infected Borans had significantly higher levels than the infected Friesian cattle ($p<0.05$).

2.3.4 Pathology

The reviews by Dawes and Hughes (1964), Taylor (1964), Boray (1969), Jones and Hunt (1983) and Losos (1986) summarise the findings reported over the years. The lesions produced by *F. hepatica*, as well as *F. gigantica* are most constant and important in the liver although occasionally the parasite may reach the lungs or other tissues where they are usually found within abscesses (Jones and Hunt 1983). Sinclair (1967) however, is of the opinion that lack of information on the changes in other organs especially intestine, is responsible for the biased view that the most important pathological changes take place in the liver.

Gross pathology

Gross pathological lesions depend on the stage and severity of the infection (Taylor, 1964; Simesen, Eriksen, Nansen, Andersen and Nielsen, 1968; Losos, 1986; Bürger, 1992). Simesen, Eriksen, Nansen, Andersen and Nielsen (1968) reported an enlargement of the liver with a mottled appearance especially on the visceral surface in chronic fasciolosis. The bile ducts are prominent and thickened although

calcification was not seen in the infected sheep. Peritoneal adhesions were common and on incision all livers exhibited defined pseudolobulation due to increased amounts of connective tissue. Dow, Ross and Todd (1967) confirmed these findings in calves followed from 3 wpi. and they also recorded liver haemorrhages due to fluke migration up to the 23 wpi in some cases. At 23 wpi. all the examined infected calves clearly showed calcification of the bile duct walls, the bile ducts were either dilated or stenosed. Taylor (1964) emphasised that calcification is usually seen in cattle and not in sheep. However in both hosts species the hyperplasia of the bile duct begins long before the flukes arrive (Simesen, Eriksen, Nansen, Andersen and Nielsen, 1968; Taylor, 1964; Bürger, 1992; Boray, 1969).

Boray (1969) gave the most comprehensive review of gross pathological changes in relation to numbers of flukes in the liver and weeks after infection. He found, for example that liver haemorrhage, rupture and haematoma, blood stained fluid in the peritoneum and oedematous lymph nodes were prominent only in fluke burdens of above 1000 per sheep and between the 4-13 wpi. On the other hand in light infections the typical haemorrhagic tracks were found mainly in the left lobe of the liver 4-5 wpi. Uzoukwu and Ikeme (1978) studied 6887 livers of Fulani Zebu cattle slaughtered at the Nsukka abattoir in Nigeria between 1975 and 1977 and of these, 15 were totally and 60 partially condemned. Gross lesions were described as contracted livers with prominent bile ducts containing the liver flukes. Also seen in one liver were kidney shaped firm and fleshy adenomatous growths.

Mahato (1993) observed after examining buffalo livers from the abattoir that two types of haemorrhages i.e petechiae and echimosis were common in livers harbouring *F. gigantica* and liver abscesses were also seen in most of these livers. Lapage (1968) included in the main pathological changes of chronic fasciolosis, anaemia, ascites, hydrothorax and hydropericardia.

Carcasses of buffaloes infected with *F. gigantica* were cachectic (little or no fat deposits), and there was severe peritonitis with serosanguinous ascitis in a calf which died 15 weeks post infection (Mahato, 1993). Although the gross pathology confined to the livers, all the visceral organs were pale. There was serous atrophy of mesenteric fat and enlarged hepatic lymph nodes. Most larger bile ducts were markedly dilated, the bile duct walls were thick and fibrotic, however were not calcified (Mahato, 1993). Ajanusi, (1994) observed no changes in rats infected with *F. hepatica* after day one, by day 14, however the livers were enlarged, friable and peritonitis was common. At day 56 all the livers were fibrotic.

Histology

Ross, Dow and Todd (1967) carried out a comprehensive histological comparison of the lesions of fasciolosis in pigs and other hosts from 1-10 weeks after infection. Pigs show milder lesions than in cattle, sheep and goats.

Singh and Parihar (1988) examined pathologically fluke infection in livers of sheep and goats. In advanced lesions, there was development of granulomas around the fluke eggs in the bile ducts and their products with calcification and foreign body reaction. The formation of granulomas around eggs and parasites products was also

reported by Simesen, Eriksen, Nansen, Andersen and Nielsen (1968) and Mahato (1993). Histologically, the flukes are seen surrounded by a layer of compressed parenchyma cells, some of which are necrotic. In the later stages, the core of the older lesions is reduced in size and is gradually replaced by macrophages and fibroblasts. Patches of the black pigment appear due to the haematin discharged by the fluke (Sinclair, 1964; Bitakaramire and Bwangamoi, 1969). Uzoukwu and Ikeme (1978) studied 6887 livers of Fulani Zebu cattle slaughtered at the Nsukka abattoir in Nigeria between 1975 and 1977. Of these, 15 were totally and 60 partially condemned. Histopathology revealed numerous micro-abscesses containing *Fasciola* larvae or eggs with accompanying cellular reactions and calcification. There was interlobular fibrosis, proliferation of bile ducts and peribiliary fibrosis in the absence of parasites and nodular hyperplastic growths of bile ductiles with complete absence of secretory ability.

Bitakaramire and Bwangamoi (1969) administered various numbers of metacercariae to 8 month old calves. Hepatic fibrosis was found in some but ascites was present in 5 calves given 10,000 metacercariae. An increase in connective tissue in portal triads coupled with slight lymphocytic infiltration was apparent in histological examination. Much of the parenchyma was replaced by fibrous tissue while bile ducts were hyperplastic. Bile ducts with flukes had denuded epithelium or showed capillary proliferation. The smooth muscle of the bile ducts was necrotic and infiltrated by mononuclear phagocytes and eosinophils

Mahato (1993) reported a mild periportal fibrosis with some mild biliary hyperplasia. The periportal inflammation comprised both mononuclear cells and eosinophils. There were haemosiderin deposits in a periportal areas in two of the twelve goats.

2.4 THE ECONOMIC IMPORTANCE OF FASCIOLOSIS

Fasciolosis is an important parasitic disease with a cosmopolitan distribution. Although more a problem of young stock, the disease is also a problem in older animals (Armour and Urquhart, 1974). It is one of the most economically important helminth diseases hampering productivity in cattle, sheep and goats in endemic areas. The disease reportedly occurs in 300 million cattle and 250 million sheep world-wide (Maurice, 1994). Besides mortality, the disease causes lowered live weight gains, poor carcass quality due to its effect on carcass composition, liver condemnations, lowered milk production, and abortions. Additional costs occur from implementation of control measures including the cost of purchasing fasciolicides.

The economic importance of fasciolosis in the livestock industry ranges from the devastating losses it causes from sudden death in the acute disease caused by migrating, immature flukes, mainly seen in sheep (Ollerenshaw, 1971) to the less spectacular effects of chronic disease seen in many hosts. Clinical disease, which is most frequently seen in cattle under two years old, is characterised by weight loss anaemia, eosinophilia and hypoalbuminaemia due to the haematophagic activities of the adult fluke in the bile ducts (Sewell, 1966; Armour, 1975).

In the USA, economic losses were first documented in the 1950s when the Department of Agriculture reported losses of several million $ US due to liver condemnations at abattoirs (Emment, 1956).

The measurement and evaluation of economic effects of diseases were considered by Morris and Meek (1980). They pointed out that evaluation of diseases in economic terms, and using this as the sole basis for deciding the economic importance and institution of control measures is misleading, since this gives the impression that the losses are recoverable by treatment. However control measures themselves cost money, thus evaluation of economic benefits of control is a more useful measure. This emphasises the monetary advantages of controlling the disease rather than the costs of no control. Such evaluations can be very expensive and the authors recommended that such undertakings should be few and provide as much information as possible. An alternative to this approach is the use of computer models in which variables can be computed within certain limits in order to predict possible outcomes (Morris and Meek, 1980).

2.4.1 Mortality in the Definitive Host

Fasciolosis is often considered as a chronic 'production' disease and referred to as 'production disease' with the economic impact solely measured by liver condemnations (Losos, 1986; Malone, Loyacano, Amstrong and Archbold, 1982). Acute and subacute fasciolosis due to *F. gigantica* can be caused even by a very light infection resulting in very high mortality especially in goats and sheep (Hammond and Sewell, 1990).

The severity and mortality depends on the level of infection, the species of the definitive host and parasite and most of all the nutritional condition the host is in (Hammond, 1965; Sewell, 1966; Boray, 1969; Hammond and Sewell, 1975; Chick, Coverdale and Jackson, 1980; Ogunrinade, 1984a; Vassilev and Jooste, 1991; Ngategize, Bekele and Tilahun, 1993).

There is very little information reported to be able to conduct a concise evaluation of the enormity of the losses caused by mortality as a result of liver fluke infection especially in developing countries (Dargie, 1986). There is, however, enough information to appreciate that mortality occurred by fasciolosis is a significant problem especially if the host is infected with *F. gigantica* (Hammond, 1965; Ogunrinade,1984a; Vassilev and Jooste, 1991).

In Nigeria, Ogunrinade and Ogunrinade (1980) estimated an annual financial loss of US$1.03 million as a direct result of mortality due to fasciolosis. In Zimbabwe, Vassilev and Jooste (1991) reported that 30 Zimbabwean dollars (Z$30) per head of cattle are lost because of deaths caused by *F. gigantica* and in Ethiopia Ngategize, Bekele and Tilahun (1993) estimated a loss of US$10.9 million on account of small ruminants mortality after being infected with *Fasciola gigantica.* Small ruminant mortality due to fasciolosis is bound to be higher in developing countries but is not fully understood.

2.4.2 Liver Condemnations

Liver fluke damages the liver of it's definitive host making it both visually unpleasant and unpalatable to eat thus leading to condemnation. Abattoir liver

inspection has long been used to estimate fasciolosis prevalence in an area or country.

Since fasciolosis is world-wide problem economic losses are a global problem. Although Dargie (1986) reported a ten year (up to 1987) reduction, from 35% to 6% and 75% to 38% in the United Kingdom and Republic of Ireland respectively, a report by Malone, Loyacano, Amstrong and Archbold (1982) shows however that the gulf coast states had about 1.4 million livers condemned due to liver fluke in 1981 alone. This was 22.4% of all condemned livers amounting to a financial loss of US$7.2 million.

The situation is even worse on the African continent. For instance Schillhorn van Veen, Usman and Ishaya (1980) were able to show that 65% of cattle 40.8% of sheep and 17.6% of goat livers were condemned because of fluke infection in their two year study in a rural abattoir in Nigeria. In Kenya an annual average of 11.5% in cattle 2.5% in sheep and 2.7% in goats of all the livers from slaughtered animals were rejected due to flukes (Anon, 1986). In the Ethiopian highlands about 51% of all liver condemnations were due to *Fasciola spp.* (Ngategize, Bekele and Tilahun, 1993). In Southern Africa the situation resembles very much that of East Africa, for instance in Zambia reported liver condemnations of between 80% and 90% (Silangwa, 1973), in Zimbabwe 43.3% of all rejected livers in 1989 were caused by *F. gigantica* according to Vassilev and Jooste (1991) and Alves (1970) in Mozambique reported 50% of all slaughtered animals harboured liver flukes in their livers .

Surveys have been conducted in many developing countries i.e. Arabian countries (Magzoub and Kasim, 1978), in the Indian Subcontinent and the South Pacific (Losos, 1986). Most recently Mahato (1993) reported that 347 buffaloes out of 408 (85%) and 14 goats out of 39 (35.9%) had their livers condemned because of liver fluke.

2.4.3 Influence of Fasciolosis in Growth and Liveweight

Liveweight gain depression and impairment of growth is a major effect of fasciolosis although, researchers have yet to establish the level of fluke burden enough to significantly depress weight gain and retard growth .

In sheep a burden as few as 45 adult *F. hepatica* showed a significant reduction of live weight gain (Hawkins and Morris, 1978). Boray (1969), Sinclair (1962) and Berry and Dargie (1978) suggested that a burden above 350 *F. hepatica* seriously reduced live weight-gain.

In cattle Hope-Cowdery, Strickland, Conway and Crowe (1977), Oakley, Owen and Knapp (1979), Chick, Caverdale and Jackson (1980), Malone, Loyacano, Armstrong and Archbald (1982), Hope-Cowdery (1984) and Mahato (1993) reported that even a very low burden (37 *F. hepatica*) is capable of impairing growth and depress weight gain. Mahato (1993) worked with both *F. hepatica* and *F. gigantica* reported similar results.

In both small and large ruminants live weight gain and growth are indirectly proportionate to *F. gigantica* or *F. hepatica* burden however other factors are also

important and including importantly the nutritional status of the definitive host (Berry and Dargie, 1976; Chick, 1980; Hope-Cowdery, 1984; Losos, 1986) .

Studying the effects of treatment against fasciolosis Sewell (1966), Hörchner, Hennings, Verspohl, Averbeck and Boch (1970) and Malone, Loyacano, Amstrong, and Archbold (1982) showed indirectly the importance of fasciolosis by comparing weight gain and production of treated and untreated herds.

There is very little information about financial loss due to depression of growth and live weight gain, however Ogunrinade and Ogunrinade, (1980), Malone, Loyacano, Amstrong, and Archbold (1982) and Vassilev and Jooste,(1991) and Ngategize, Bekele and Tilahun, (1993) were able to include this aspect in their calculation to estimate total loss incurred by fasciolosis and were able to show that it accounts for a significant loss.

2.4.4 Wool Growth and Quality

Reduction in wool growth and quality are common features in helminth infections. Reduction in wool growth is usually associated with adult *Fasciola spp.* burden and with as low in number as 30 flukes (Dargie, 1986). As with suppression of liveweight gain there is a correlation between adult liver fluke burden and the wool quality. Edward, Al-saigh, Williams and Hope-Cowdery (1969) and Reid (1978) showed that treatment of infected ewes results in improvement of both quality and quantity of fleeces. It is generally agreed that if the treatment is effective and the nutrition correct, one should be able to restore the pre-infection wool growth level but the gross losses remain until next shearing (Hope-Cowdery, 1984). Mean wool

quality which is measured by determining fibre diameter length and tensile strength remains poor, resulting in low grade fleece and financial losses (Hope-Cowdery, 1984; Dargie, 1987).

2.4.5 Milk Production

The dairy industry is under developed in most tropical and sub-tropical countries but regardless of the location any suppression of milk production will affect production.

Hope-Cowdery (1984) showed that *Fasciola* infection reduces milk production. Ross (1970), Hörchner, Henmings, Versopohl, Averbeck and Bock (1970), Randell and Bradley (1980) proved that treating infected cows with Oxoclozanide greatly improved milk total solids thereby increasing milk quality leading to financial benefit for the farmer.

There are severe implications to new-born stock in milk quality or quantity reduction (Sinclair, 1972; Crossland, Johnstone, Beaumont and Bennett, 1977; Reid and Armour, 1978; Hope-Cowdery, 1984) who reported poor live weight gain of lambs from ewes infected with *Fasciola spp.*

2.4.6 Reproduction Effects

The effect of liver fluke infection on reproductive capacity of livestock may be the least researched aspect of this disease. Hope-Cowdery (1971) suggested that fasciolosis reduces performance in ewes when the invasive stage of parasitism coincides with mating and establishment of the foetus. However a number of authors

have reported that infection with liver fluke does have a significant effect on the fertility in food animals (Oakley, Owen and Knapp, 1979; Hope-Cowdery; 1984; Dargie 1986; Kurma and Sharma, 1991; Vassilev and Jooste, 1991; Ngategize, Bekele and Tilahun, 1993).

Kurma and Sharma (1991) in their survey found that only 36% anoestrus were observed in uninfected cows but 50% anoestrus in cows with chronic fasciolosis. Heavy *Fasciola* infection in sheep has been associated with abortions, stillbirths and lambs of low birthweight followed by reduction in liveweight gains (Sinclair, 1972; Dargie, 1986). It has also been reported that calves may be born infected (Rees, Sykes and Rickard, 1975).

2.4.7 Feed Utilisation

A reduction in voluntary feed intake is a common feature in most helminthosis including fasciolosis. The magnitude of the effect is related to the severity and duration of the infection and the level of protein intake (Boray, 1969; Berry and Dargie, 1976). There is very little data backing these facts especially because of inaccuracies in feed intake measurements and the bias arising from intake differences between infected and non-infected animals (Hope-Cowdery, Strickland, Conway and Crowe 1977; Oakley, Owen and Knapp, 1979; Dargie, 1989). Sykes, Coop and Rushton, (1980) reported a 15% drop in feed intake in an infected group as compared with the uninfected control.

Changes in the efficiency in production results from reduction in feed intake, digestibility and of nutrients. Weight loss leads to reduction of nitrogen retention as

assessed by measurements of body composition (Sykes, Coop and Rushton, 1980) or nitrogen balance (Dargie, Berry and Parkins, 1979). Sykes, Coop and Rushton (1980) found that in sheep this occurs with a burden of more than 230 adult *F. hepatica*. However, an adult fluke burden of 87 did reduce body fat deposition, protein content and gross efficiency of metabolisable energy used for growth.

2.5 CHEMOTHERAPUETIC TREATMENTS FOR FASCIOLOSIS

The efficiency of a fasciolicide should ideally be measured against its ability to cure most important *Fasciola* species from immature to mature stages. At the same time these drugs should not be toxic to most host animals and their form should enable easy application to large numbers of animals in the field. It is the continuous demand for drugs with these qualities that has given rise to the number of anthelmintics now found on the market.

The earliest fasciolicides used were mainly chlorinated hydrocarbons especially carbon tetrachloride, hexachlorethene and hexachlorophene (Gibson, 1969 and Losos, 1986). These fasciolicides are however no longer widely used.

There are now a wide range of fasciolicides some more commonly used than others, and differ from region to region (Troncy, 1989). Berger (1971) compared the therapeutic activities of five fasciolicides against immature and mature *F. gigantica* in experimentally infected 2½ to 5 month old calves. He was able to register high efficiency of hexachlorophene, nitroxynil and bilevon-R at doses of 15, 10 and 3 mg/kg respectively. Oxyclozanide (Zanil®) at the recommended dose of 10 mg/kg

given orally showed little effect. It however was effective against four week old flukes at 20 mg/kg. At the maximum dose nitroxynil (40 mg/kg) and carbon tetrachloride (0.4 mg/kg) were unable to cure four week old *F. gigantica* infection in cattle. Roy and Reddy (1969) in India recorded 100% efficacy against six week old flukes when they used 10 mg/kg nitroxynil in cattle, water buffalo and sheep.

Rafoxanide is another commonly used fasciolicide (Troncy, 1989; Losos, 1986). The therapeutic efficacy of rafoxanide against *F. gigantica* infection in cattle has been carried out by Snijders, Horak and Louw (1971) who found it effective at 3.75 mg/kg against all adult flukes and Snijder, Louw and Errano (1971) concluded that a 2.5 mg/kg to 20 mg/kg range is not only effective against adult flukes but also non-toxic in cattle.

Boray, Crowfoot, Strong, Allison, Schellenbaum, Von Orelli and Sarasin (1983) found that triclabendazole given at a dose of 2.5 mg/kg was only 53% effective against four week old flukes but 5 mg/kg cleared 92% of the four week old infection. This dose is in fact half the recommended therapeutic dose. Maes, Vanparijs, Lauwers and Deckers (1990) tried to compare the viability of fluke eggs shed by animals treated with closantel or triclabendazole (10 mg/kg) in sheep. Although the trichlabendazole was 94.2% effective and closantel 83% against fasciolosis, the eggs from closantel treated animals had only 37% embryonated eggs while triclabendazole group had 71% much closer to 82% found in a non-treated group. Many authors believe that triclabendazole is the most effective broad spectrum fasciolicides (Richards, Bowen, Essenwein, Steiger and Bücher, 1990;

Losos, 1986; Troncy, 1989; Hammond and Sewell, 1990; Bürger, 1992). Waruiru, Wada and Munyua (1994) reported that a single dose of 12 mg/kg of triclabendazole and 13.5 mg/kg of oxyclozanide reduced egg shedding by more than 91% but both flukecides were less effective against immature flukes. The current fasciolicides are shown in table **2.3.**

In Australia the first comprehensive case of drug resistance was reported by Boray and De Boso (1989) involving mainly salicylanilide, rafoxanid and closantel but failed to find resistance against triclabendazole and the sulphonamide, clorsulon.

Table 2.3: Anthelminthics reported to be effective against *Fasciola* spp. in both small and large ruminants with variable efficacy against different stages of flukes. (4 = ineffective, 3 = less than 40 % effective, 2 = above 60% effective and 1 = above 85% effective)

			Efficacy against both *Fasciola species*				
Chemical group	Drug	Standard dose	Adult	4-8 wks	1-4 wks	Notes	Source
Aromatic amid	Diaphenethide	100 mg/kg	3	4	4	Not used in cattle because of higher dose needed for 8 wks and over	Blood (1994) Bürger (1992) Blood *et al.* (1989)
Benzimendazoles	Albendazole	10-15 mg per kg	4	1	1		Boray *et al.* (1983) Eckert (1989) Güralp and Tinar, (1984)
	Triclabendazole	10 mg/kg	4	4	4		Eckert (1989) Richards *et al.* (1990) Fawcett (1990)
Chlorinated hydrocarbons	Carbon tetrachloride	0.1 ml/kg 1ml	4			Too toxic to use in cattle	Blood (1994) Boray (1969)
	Hexachloroethene	10 g/50 kg	4			For sheep 15-8 g/adult animal	Gibson (1969) Boray (1969)
	Hexachlorophene	15 mg/kg oral	4				Richards *et al.*(1990)
Salicylanilides and substitute phenols	Bromsalans		4	3	1		Bürger (1992) Blood (1994)
	Brotianide	5 mg/kg	4	4	2	For 6 week old flukes	Bürger (1992)

Table 2.3 (contd).

			Efficacy against both *Fasciola species*				
Chemical group	Drug	Standard dose	Adult	4-8 wks	1-4 wks	Notes	Source
	Closantel	10 mg/kg					Maes (1990) Troncy (1989)
	Oxyclozanide	10 mg/kg P.O.	4	1	1	15 mg/kg for sheep and goats	Troncy (1988) Berger (1971)
	Niclosamide	15 mg/kg	4	3	1		Gibson (1969) Bürger (1992)
	Nitroxynil	S/C 8-12 mg/kg	4	3	1	For immature flukes 15 mg/kg	Bürger (1992) Troncy (1981)
	Rafoxanid	5 mg/kg (7.5-10.5 mg/kg)	4	4	3	7.5 to 10 mg/kg is mostly recommended for immature flukes	Snijders (1971) Bürger (1992)
Sulphonamide	Clorsulon	20 mg/kg	4	4	3	Not available for small ruminants	Troncy (1985) Bürger (1992)

2.6 CURRENT CONTROL METHODS FOR FASCIOLOSIS

The rationale of fasciolosis control measures depends very much on the understanding of its epizootiology (Hiepe, Buchwalder and Ribbeck, 1981; Bürger, 1992). Over (1982) stated it that the knowledge of the ecology of the parasites holds the key to the success of the control measures. This knowledge unfortunately is lacking in many developing countries, mostly tropical and sub-tropical countries where the disease is endemic. The other main factor according to Hammond and Sewell (1990) is the economic feasibility of the measures being considered.

There are numerous ways in which control measures have been classified by different authors (Taylor, 1964; Malek, 1980; Bürger, 1992), but they can be divided into two categories according to their aim. The first class includes those measures targeted at the definitive host with an aim of either reducing the adult fluke burden hence pasture fluke egg contamination or reduce the new infection becoming a clinical syndrome (Hiepe, Buchwalder and Ribbeck, 1981; Blood, 1994; Urquhart, Armour, Duncan, Dunn and Jennings, 1996). The second group of measures is directed towards the intermediate host, the amphibic or aquatic snail, leading to reduction in metecercariae available to infect the host.

2.6.1 Strategic Use of Anthelmintics

One of the comprehensive reviews of this subject was by Taylor (1964) and already at that time the importance of anthelmintics for control of fasciolosis was underlined. The determining factor of using anthelminthics for control is the

understanding of the local disease dynamics. This helps to determine the times that dosing can take place in order to produce maximum effect. Whitelaw and Fawcett (1977), Bürger (1992) and the report by Urquhart, Armour, Duncan, Dunn and Jennings (1996) suggested that the best time for strategic control of *F. hepatica* in sheep and calves in UK are as detailed below:

1) April to early May, treatment of adult sheep with any fasciolicide effective against adult parasites to reduce pasture fluke eggs contamination.
2) Early October, dose the whole flock with a drug effective against liver parenchyma migratory stages such as triclabendazole.
3) January, treat with any anthelminthic effective against adult liver flukes.
4) If the year is extra wet, one or two more doses can be given with one 6 weeks after the April/May and another one 4 weeks after the early October dose.

Control of fasciolosis caused by *F. gigantica* is mostly adapted from the measures developed for fasciolosis caused by *F. hepatica*. In Thailand according to Muagyai (1989) buffaloes older than 8 months are treated in early September to prevent snail contamination with those in poor conditions getting a second dose in April or May especially in highly endemic areas. In West Africa it is generally advised to treat cattle either with Nitroxynil or Rafoxanide at the beginning of the dry season for exposed animals. A repeat dose at the end of the dry season or beginning of the wet season to reduce pasture contamination (Troncy, 1989).

In general prophylactic treatment is directed towards reducing fluke burden at the time when the parasite is susceptible to available drugs and when the nutritional

status of the animals is at its lowest (Bürger, 1988; Troncy, 1989; Urquhart, Armour, Duncan, Dunn. and Jennings, 1996).

2.6.2 Chemical Control of Vector Snails

Molluscicides are effective in controlling snails, but the high cost and environmental impact renders them unsuitable for use in many countries, for example in the USA their use is unwelcome in most circumstances (Simpson, Kunkle, Courtney and Shearer, 1985; Westcott and Foreyt, 1986).

There are many molluscicides, which although not in common use, are effective and might be employed in the future, i.e. insoluble copper compounds such as copper pentachlorophenate, copper carbonate and cuprous oxide, Yurimin (P-99) a Japanese product (Harada, 1974).

The efficacy of individual molluscicides may differ from one snail species to another. Boray (1969) for example, using adults and their eggs found that *L. tomentosa* required a higher concentration of Sodium Pentachlorophenate (NaPCP) compared to Niclosamide, N-tritylmorpholine (Frescon) and copper sulphate.

The efficacy of four molluscicides NaPCP, (Frescon), copper sulphate, Bayluscide® and Yurimin on *L. ollula* was tested and it was found that Bayluscide (Niclosamide) the most effective of them all (Harada, 1974).

The application of molluscicides according to the European experience should be targeted to kill the snails prior to the commencement of breeding in May or in Summer (July/August) to kill infected snails. The spring treatment may become

difficult because of limitation in pasture growth making vehicular access difficult (Hiepe, Buchwalder and Ribbeck, 1981; Urquhart, Armour, Duncan, Dunn, and Jennings, 1987). The same strategy of killing snails shortly before shedding the cercariae can be adapted for aquatic snails but application of molluscicides in water is environmentally unacceptable therefore not a practical alternative (Hammond and Sewell, 1990).

2.6.3 Use of Molluscicides Extracted from Plants

There is evidence that some plants or their products are toxic to the snail vectors of trematodes and could be used as molluscicides (Kloos and McCullough, 1981) and Hammond (1970) reported that it was difficult to find *L. natalensis* in Kenya in areas where *Eucalyptus* trees grow. No experimental observations were made however and this prompted studies to determine the effect of *Eucalyptus* leaves on aquatic snails in Kenya. Leaves of *Eucalyptus globulus*, a widely distributed species in Kenya, killed 100% of *L. natalensis*, *Biomphalaria* spp. and *Bulinus* spp. at 1.5 g/l in 24-72 hours. Other species with molluscicidal properties were *Eucalyptus alba*, *Eucalyptus robusta*, *Eucalyptus microcorys* and *Eucalyptus melliodora* but these are of limited distribution in the country (Cheruiyot and Wamae, 1988).

Hammond, Fielding and Nuru (1994) observed that the use of plant molluscicides is a labour intensive undertaking and would require skilled labour. They suggested a solution would be to identify plant species with a 'self-delivery system' such as *Eucalyptus* spp. Such plants could be planted along the habitats so

that their leaves will fall in the water and kill the snails. Thus providing a self-sustaining snail control and by implication control against fasciolosis.

Another plant with molluscicidal properties is *Phytolacca dodecandra* (Lemma, 1970). *Phytolacca dodecandra* (L'Herit), is commonly known as soap berry and its dried berries (endod) are widely used in Ethiopia as a soap substitute. In natural bodies of water where 'endod' was used high snail mortalities were seen (Lemma, 1970) and testing of various plant parts indicated that the berries were the most potent source of the molluscicide. The activity of 'endod' was preserved over a wide range of water pH, temperature, under ultraviolet radiation and also in the presence of river bed mud. It had an LC_{90} of 20 ppm in its crude form and thus offers an alternative and natural method for snail control.

2.6.4 Biological Control

Environmental considerations have to be taken into account when formulating snail control measures. Usually molluscicides are applied in water bodies without due consideration to other aquatic fauna. This means that non-target organisms might be exposed to harmful effects by the molluscicides, thereby upsetting the balance of nature. Copper sulphate, for example, is toxic to both livestock and fish when applied as a molluscicide at 0.1-0.2 ppm (Soulsby, 1982). Even if no immediate deleterious effects might be apparent, repeated applications might have such effects. Draining of marsh lands can also be used to control snails but with an obvious detrimental effect on the balance of the marsh land ecosystem.

There have been more reports on the biological control of *Schistosoma*-bearing snails than the *Fasciola*-bearing ones. It is found that most biological control methods recommended experimentally have failed to succeed in field trials (Boray, 1969; Malek, 1980).

Berg (1969) attempted to use fly larvae, *Dichaetophora biroi*, as a killer of the snails, but the field trials failed. The use of other snail predators such as birds (ducks), frogs and fish have been reported (Hammond and Sewell, 1990; Blood, 1994). Boray (1964) considered the use of larvae of trematodes which live as true parasites of the snails thereby helping to kill the snail, however, further investigation disclosed that the death of the snails takes place after the life-cycle of flukes in the snail is completed i.e. shedding of metacercariae. Overall very little progress has been made in the use of biological methods as a control measure for fluke-bearing snails.

2.6.5 Control by Environmental Manipulation

Control by farm management and environmental manipulation of the snail environment is arguably the cheapest method of avoiding fluke infection in areas where the eco-system permits. The following physical control measures have been recommended by Taylor (1964), Boray (1969), Soulsby (1982), Hammond and Sewell (1990) and Bürger (1992). These recommendations however are not practical in regions such as Bangladesh where flooding leaves vast areas of suitable areas.

- Drainage poses a solution but cost is a major constraint and this method is only suitable for the control of amphibic snails.

- Fencing snail infected areas. This is mostly not practical if the infested area is the only water hole in the area. The cost of fencing these areas has to be considered.
- Clearing plants and building concrete or stone platform to avoid animals grazing in and around the water source.
- Adequately maintained troughs where water is provided by pipes from the source, boreholes, deep wells or mobile water sites does prevent host-parasite contact (if there are no snails in the troughs).
- Pasture rotation is only possible under commercial production systems or when there is adequate pasture.

2.7 IMMUNOCHEMISTRY OF *FASCIOLA* SPP

2.7.1 Cross-reactivity of *Fasciola spp.* Antigens

Immunodiagnosis in helminthosis including fasciolosis has been hampered by lack of species and/or stage-specificity in the reagents used manifested through cross-reactivities with other helminths (Kagan, 1979; Hillyer and De Aleca, 1980, Fagbemi and Obarisiagboni, 1991; Rodriguez-Perez and Hillyer, 1995).

Cross-reactions occur between *Schistosoma spp.* and *Fasciola spp.* in farm animals (Christensen, Monrad, Nansen and Frandsen, 1980; Hillyer, 1985; Haroun and Hillyer, 1988). Rodriguez-Perez and Hillyer (1995) reported *Fasciola spp* extracts that cross-react with *S. mansoni* but not the reverse. Hillyer (1979) immunised mice with crude extracts resulting in 28-58% reduction in *S. mansoni* burden following challenge infection. After purifying these extracts by

immunosorbent chromatography in immunised mice he managed to reduce the *S. mansoni* burden. Chapbell, Kelly, Townsen and Dineen (1977) showed that a primary infection with *Taenia hydatigena* cysticerci, for 12 weeks in sheep generated a 95% protection against challenge with *F. hepatica*. This was confirmed by Dineen, Kelly and Capbell (1978). Michel and Amour (1981) reported that neither *N. braziliansis* nor *T. hydatigena* cysticerci induced resistance to a challenge with *F. hepatica*. Hughes, Harness and Doy (1978) also failed to demonstrate resistance to *F. hepatica* challenge infection in sheep, goats and cattle after a primary infection with *T. hydatigena* cysticerci.

Cross-reactions of *Fasciola* spp. with other helminths like *Schistosoma*, *Clonorchis*, *Paragonimus* and *Paramphistomum* have been reported in humans (Hillyer and Capron, 1976). Partial purification of this antigen using Sephadex G-200 was however able to eliminate most of this cross-reactivitiy. Hillyer and Capron (1976) using crude *F. hepatica* extracts found an extensive cross-reaction with hydatidosis, trichinosis, cysticercosis and amoebic hepatitis patients. However, after partially purifying the extracts with Sephadex G-200 chromatography cross-reactions were reduced without diminishing the sensitivity.

Choi and Lee (1979) found that despite the fact that the three protein peaks from a Sephadex G-200 chromatography purified adult fluke extracts contained specific proteins the other fractions cross-reacted with sera from paragonimus, paramphistomum and clonorchis infected hosts. A comparison by Fagbemi and Oberisiagbon (1991) of whole crude and Sephacryl S-300 column chromatography

semi-purified parasite extracts from three trematodes, *F. gigantica*, *S. bovis* and *D. hospes* identified cross-reactivity in both crude and semi-purified extracts confirming the findings by Hillyer and Serano (1986) who reported common antigens in *S. mansoni* and *Paragonimus westermani* adult worm extracts.

2.7.2 Characterisation of *Fasciola spp.* Antigens

Because of the problems of cross-reactivity associated with the use of crude parasite extracts in the immunodiagnosis of fasciolosis, many studies have been conducted with the aim of identifying and characterising potentially *F. hepatica*-specific excretory and secretory products (E/S), surface or somatic antigens and to a lesser extent *F. gigantica.* Methods employed include column chromatography, radio-immunoprecipitation assays, immunoelectrophoresis, western blotting and Enzyme-linked Immunosorbent Assay (ELISA) (Hillyer and Santiago 1977, Zimmermann and Clarke 1986, Pantelouris, 1965; El.Bahi, Malone, Todd and Schnoor, 1992).).

There are methods of value in immunodiagnosis such as Western blotting techniques and the high sensitivity of immunological methods like ELISA. The Western blotting techniques provide a unique tool to simultaneously compare parasite-specific and cross-reacting antigens (Tsang, Peralta and Simons, 1983).

Using Western blot, Marrero, Santiago and Hillyer (1988) analysed the Sephadex G-75 polypeptides fraction and found that molecules ranging from 25-30 kDa could be used to diagnose *F. hepatica* infection in rabbits, cows and sheep. Hillyer and Soler de Galanes (1988) were able to separate between two polypeptides,

a specific 17 kDa and the one cross-reacting with *S. mansoni* and *T. spiralis* infection (63 kDa) by using the Western blotting.

Western blotting or Enzyme-linked Immunoelectrotransfer Blot (EITB), as it is also called, can improve the specificity of immunodiagnosis by comparing reactions with other parasite infections (Santiago and Hillyer, 1986; Solano, Ridley and Munocha, 1991; Poitou, Baeza and Boulard, 1992; Keegan and Trudgett, 1992).

In a study using Western blotting 3h ES of *F. gigantica* was characterised using infected sheep sera and antigens of 43-75 kDa were detected by 2 weeks post infection and more antigens were reportedly recognised with time. A 69 kDa antigen was thought to be of diagnostic value since it was recognised 2 weeks post infection. An 87 kDa antigen which was recognised by 12 wpi. and lost 2 weeks post-chemotherapy was thought to be of value in assessing chemotherapeutic success (Guobadia and Fagbemi, 1995).

Detection of circulating antigens has been reported and an 88 kDa antigen derived from adult *F. gigantica* was used to diagnose experimental and natural infections in cattle using a double polyclonal antibody ELISA. Although the antigen was of some value, there were cross reactions with 18.8% of *Paramphistomum* infected cattle. The assay gave negative absorbence values (below cut-off) three weeks after oxyclozanide treatment while as might be expected antibody assays were still positive 6 weeks after chemotherapy (Fagbemi, Obarisiagbon and Mbuh, 1995). In a related study monoclonal antibodies were produced against a 28 kDa protease purified from adult *F. gigantica* E/S which recognised the 28 kDa protease using a

Falcon assay screening test-ELISA (FAST-ELISA), Western Blot and Immune Precipitation (Fagbemi, 1995). Monoclonal antibodies had been produced against a 27 kDa protease of a *Fasciola* spp. by Yamasaki, Aoki and Oya (1989) while Solano, Ridley and Minocha, (1991) produced monoclonals against ES components of *F. hepatica*.

Sephacryl S-200 column chromatography was used to separate adult *F. hepatica* somatic extracts into four fractions. A 6 kDa fraction, showing no cross-reactions with *S. mansoni* infected rabbits serum on Counter Immunoelectrophoresis (CIEP) was used (Hillyer and Santiago, 1977). These authors, repeated their experiments with Albino mice (Hillyer and Santiago, 1981). Sinclair and Wasall (1988) were able to diagnose fasciolosis in cattle after fractionating the *F. hepatica* extract on a column of Sephacryl S-300. After comparing Sephacryl S-200 and Superose-6 (FPLC) colunm chromatography, Zimmerman and Clark (1986) concluded that the FPLC system seemed to be faster and gave a higher resolution separation of antigen. Santiago and Hillyer (1986) fractionated *F. hepatica* homogenate on Sephacryl S-200 and with Western blot identified antigenic polypeptides of which 18-23 kDa MW was recognised by infected cattle.

Lehner and Sewell (1980) using Sephadex G-200 to partially purify adult *F. hepatica* E/S products showed that molecules in the range of 100-500 and 25-50 kDa were recognised as immunogenic by infected sheep. Mansour, Youssef, Mikhail and Boctor (1983) fractionated adult *F. gigantica* somatic extracts using the Sephadex G-200.

Irving and Howell (1982) precipitated three polypeptides of MW 24, 26 and 27 kDa from ^{35}S-methionine radio-labelled juvenile *F. hepatica* E/S. After biosynthetically (^{35}S-methionine) radio-labelling, Dalton, Tom and Strand (1985) noted that molecules of between 25-30 kDa were reactive with sera from rabbits from 3 weeks after infection with *F. hepatica* . Santiago, Hillyer, Carcia-Rosa and Morales (1986) identified, after co-precipatation of ^{35}S-methionine radio labelling *F. hepatica* E/S antigen, a 33 and 62 kDa components which reacted with sera from 3-5 weeks infected rabbits. In addition at least five other major polypeptides of 120, 84, 58, 52 and 39 kDa MW were recognised by the infected sera from 6 weeks post-infection. The 29-31 kDa MW range were identified by Sexton, Milner and Campbell (1991) after biosynthetically radio-labelling of adult *F. hepatica* E/S products as the most immunogenic and most likely to be successfully used in speciation.

Studies on adult *F. hepatica* messenger RNA *in vitro* translation products, using 4 weeks post-infection sheep sera, showed that the sera recognised polypeptides in the 30-35 kDa range (Irving and Howell 1986) .

2.7.3 Defined *Fasciola spp.* Antigen

Glycocalyx turnover or replacement in newly excysted juvenile (NEJ) *F. hepatica* flukes has been suggested by Hanna (1980) as a possible mechanism for protection against the host immune responses.

The rapid discard of the surface glycocalyx, and its continual replacement by glycoproteins secreted within T1 and T2 granules, are believed to be due a

mechanism by which *F. hepatica* discards bound antibody and thus avoid immune attack (Hanna, 1980). The search for vaccine candidate or immunodiagnostically stable antigen has for a long time directed the efforts of scientists towards proteolytic enzymes (Howell, 1979)

Cathepsin L-like protease

During incubation in the presence of immune serum this glycocalyx is continuously shed and replaced, and it has been postulated that the lack of *in vitro* killing of *F. hepatica* NEJ's is due, in part, to the rapid turnover of this highly antigenic layer (Duffus and Franks, 1981) preventing the attachment of both antibodies and/or cells. After *in vitro* studies Chapman and Michell (1982) reported that immature *F. hepatica* release a papain or Cathepsin-B-like proteolytic enzyme which partially cleaves immunoglobulins in mouse, rat, rabbit and sheep this in turn could render one of the host difference systems less affective. It has been shown that the migratory liver fluke stage secretes a proteolytic enzyme which allows the parasite to survive immune attack despite being in direct contact with the host fluids (Chapman and Mitchell, 1982). This was found to be because this enzyme is capable of cleaving host immunoglobulin G and M in the way Cathepsin B or papain does. This secretion of proteolytic enzymes were initially studied by Locatelli and Paoletti (1969). Rege, Herrera, López and Dresde (1989) described one major cysteine protease from adult worms, which. has been suggested to be involved in nutritional and/or evasion of the immune response which they suggest is capable to digest haemoglobin, collagen and IgG. Smith, Dowd, Heffernan, Robertson and Dalton,

(1993) and Carmona, Dowd, Smith and Dalton (1993) demonstrated that a protease secreted by *F. hepatica* plays an important role in immunoevasion by releasing the Fc portion from IgG and thereby preventing host effector-cell attachment to newly existed juvenile flukes (NEJs). In recent years Smith, Dowd, McConigle, Keegan, Bennan, Trudgett and Dalton, (1993) reported the purification of a cysteine protease secreted by adult *F hepatica*, the first Cathepsin-L like enzyme to be identified in parasite trematodes. Later, Smith, Dowd, Heffernan, Robertson and Dalton, (1993) demonstrated that this *F hepatica* Cathepsin-L like protease plays an important role in immunoevasion by releasing the Fc portion from IgG and thereby preventing host effector-cell attachment. From their results Carmona, Dowd, Smith and Dalton (1993) suggested that Cathepsin-L cysteine protease is the major protease secreted by newly excysted juveniles (NEJ) and demonstrated that this enzyme cleaves immunoglobulin in the hinge region and prevents the *in vitro* antibody-mediated attachment of eosinophils to the NEJ.

In recent years investigations, Smith, Dowd, McConigle, Keegan, Bennan, Trudgett and Dalton, (1993), using gel filtration followed by ion exchange chromatography, reported the purification of a cysteine protease from adult ES products. By determining the N-terminal sequence, the purified protease was characterised as a Cathepsin-L like enzyme. This Cathepsin-L like enzyme molecule appears to have the molecular weight of 27 kDa, as determined by SDS-PAGE under reducing conditions. This proteolytic enzyme was classified as a Cathepsin-L cysteine protease based on many physico-chemical properties in common with

mammalian Cathepsin-L proteases, whose role is the degradation of proteins in lysosomes (Barrett and Kirschke, 1981).

Immuno-localisation studies at the electron microscope level reveal that this Cathepsin-L cysteine is packaged in vesicles within the gut epithelial cells of the parasite. If these vesicles are secretor granules, it is possible to presume that during their migration through the host-liver, the flukes secrete the Cathepsin-L cysteine protease into the host. The molecule could, therefore, protect the NEJ's from immune attack, since the enzymes are capable of cleaving host immunoglobulins (Smith, Dowd, Heffernan, Robertson and Dalton,. 1993), thus preventing antibody-mediated attachment of eosinophils to the NEJ (Carmona, Dowd, Smith and Dalton, 1993).

Proteolytic enzymes are an important functional antigen of the parasitic helminths. McKerrow, Brindley, Brown, Gam, Staunton and Neva (1990) for example identified a protease produced by *Strongyloides stercoralis* when penetrating the skin and migrating through the tissues of the host. A number of proteases have been reported to induce immune responses which could be exploited for vaccine development or immunodiagnosis (Zerda, Dreeds, Damian and Chappel, 1987; Chappel and Dresden, 1988 and Fagbemi and Hillyer, 1992).

Fagbemi and Hillyer (1991, 1992) purified and characterised a 28 kDa cysteine protease of *F. gigantica* adult worm which enhanced the sensitivity of immunodiagnosis of fasciolosis. However they do not have conclusive results as to its stability.

This enzyme is capable of cleaving host immunoglobulin G and M in a Cathepsin B- or papain- like manner. Later, Dalton and Heffernan (1989), demonstrated that *in vitro,* immature and mature *F. hepatica* release 11 distinct proteolytic enzymes.

Glutathione S-Transferase

Glutathione S-transferases (GSTs; EC 2.5.1.18) are a group of multifunctional proteins that assist the body in the detoxification of a large range of exogenously derived compounds (xenobiotics) and endogenously derived toxic compounds such as bilirubin and haem using enzymatic and binding activities (Mannervick, 1985). They are capable of detoxifying these foreign substances by conjugating reduced Glutathione to electrophilic centres of various molecules thus protect parasites from membrane damage (Mitchell, 1989).

These enzymes are widely distributed in mammals but they have also been found in abundance in tissues of adult *F. hepatica* i.e. in cytoplasm of parenchymal cells, in the subtegument and tegument and on the lamellae of the intestinal epithelium (Howell, Board and Boray, 1988 and Wijffels, Sexton, Salvatore, Pettitt, Humphris, Panaccio and Spithill, 1992). GST as a vaccine candidate for ruminants (Sexton, Milner, Panaccio, Weddington, Wijffels, Chandler, Thompson, Wilson, Spithill, Mitchel and Campbell, 1990). In this trial, sheep were challenged with 500 metacercaria and after immunisation with GST an overall reduction in worm burden of 57% was found. Thus, demonstrating a high level of protection in the vaccinated sheep. This suggests that the target of the immune attack could be GST in the

metacercariae and/or in the NEJ resulting in the subsequent damage and elimination of the parasites.

The main role of mammalian just like parasite GST is to protect the cell against immune-mediated lipid peroxidation. Thus inhibition of this enzyme offers possibilities of combining immunotherapy and chemotherapy (Brophy and Barrett, 1990).

One of the latest purified antigenic GST is the *F. hepatica* glutatione-S-transferase (FhGST) isolated from adult worms by glutathione agarose affinity (Hillyer, Galances and Battisti, 1992). This enzyme, 28 kDa MWt, has been used to evaluate whether different animals infected or immunised with *F. hepatica* or *Schistosoma mansoni* developed antibodies to this affinity- purified FhGST. These authors have found that sheep and rabbits infected with *F. hepatica* developed anti-FhGST antibodies detectable by ELISA as early as 2 weeks of infection (thus called responders) whereas neither mice nor calves equally infected with *F. hepatica* did (thus they are called non-responders).

2.8 DIAGNOSIS OF FASCIOLOSIS

2.8.1 Conventional Methods

Diagnosis of fasciolosis is still mainly dependant on the clinical observation and detection of eggs in faeces. The most prominent clinical signs, especially in small ruminants, include anaemia with submandibular oedema, lethargy, sometimes jaundice, hyperalbuminaemia and eosinophilia. Acute fasciolosis may result in death

especially in sheep (Sinclair, 1967; Boray, 1969; Sykes, Coop and Rushton, 1980; Bürger, 1992).

Coproscopic analysis is the routine method for diagnosis in patent fasciolosis. Morel (1987), after comparing sedimentation and flotation techniques and their modifications concluded that sedimentation as described by Hinaidy, Keferböck, Pichler and Jahn (1988) was very efficient though laborious and time consuming. He therefore recommended use of the differential flotation described by Hammond and Sewell (1972) in field routine work. The worst results were recorded after using flotation technique (without using saturated salt solution) as recommended by the Anon (1977).

Although most commonly used, coproscopic methods have many disadvantages. Düwell and Rusenlecter (1984) in agreement with Boray (1969) could not find any uniformity and consistency in low, medium and high worm burdens after faecal egg counts. Fluctuations in faecal egg count per gramm (EPG) is understandable considering the numerous pathophysiological changes including peribiliary fibrosis, granulation or calcification of the liver which could easily lead to obstruction of the egg passage (Boray, 1969; Malek, 1980; Urquhart, Armour, Duncan, Dunn, and Jennings, 1996). Dorsman (1957, 1962) on the other hand reported that there were relatively small variations from day to day and that the EPG was consistently highest in the early afternoon.

Unfortunately, as a diagnostic method the faecal egg count can only detect patent infections and yet most damage to the animal is done before patency by the

migrating parenchymal forms of *Fasciola* spp. (Troncy, 1989). Another limitation to the zinc sulphate or sedimentation methods of diagnosing this disease is that the numbers of eggs detected does not reflect parasite burden in the animal. The egg counts rise rapidly by 7-9 wpi. but become variable thereafter and may even be very low for a heavily infected animal (Sewell, 1966). Although these tests, if successful, when combined with epidemiological findings provide unequivocal evidence of infection, they are limited, specially when applied to prepatent infections or low-level infections (Losos, 1986; Bürger, 1992).

Serum enzyme detection

One approach has been to measure liver function enzymes since damage to the liver by the flukes causes increased seepage of these enzymes into the circulation. It is clearly possible to suggest prepatent fasciolosis among other liver damaging diseases by marked increase in serum activities of the liver enzyme, aspartate aminotransferase (AST) and glutamate dehydrogenase (GLDH). This is associated with liver parenchymal cell damage by invading young flukes. Increased serum gamma-glutamyltransferase (γ-GT) activities on the other hand is an indicator that the flukes are penetrating or already are in the bile duct. This usually occurs from about 80 days after *F. hepatica* infection (Berry and Dargie, 1976; Sykes, Coop and Rushton, 1980; Ferre, Barrio, Gonzalez-Gallego and Rojo-Vazquez, 1987).

Reid, Urquhart and Jennings, (1970) reported that the intensity of these changes is indicative of intensity of infection and host susceptibility a fact that may be of use in epidemiological surveys.

Simesen, Nielsen and Nansen (1973) also studied serum levels of γ-GT in cattle infected with *F. hepatica* and observed that the activities in serum increased one month after infection and peaked at 5 months. In a similar study Anderson, Berret, Brush, Herbert, Parfitt and Patterson (1977) performed serial enzyme assays in cattle harbouring *F. hepatica* and concluded that the two enzymes, GLDH and γ-GT, were the best indicators of acute fasciolosis. Rowlands and Clampitt (1979) also came to conclusion that the two enzymes, GLDH and γ-GT, were the best indicators of liver damage in cattle and sheep infected with *F. hepatica*.

Although earlier studies were confined to *F. hepatica*, *F. gigantica* infections have also been examined. Kumar, Maru and Pachauri (1982) working with buffaloes infected with *F. gigantica,* reported increased serum enzymes activities and an increase in serum enzyme activities in *F. gigantica* infections was shown in experimentally infected desert sheep 5 wpi. (El-Samani, Mahmoud, Fawi, Gameel and Haroun, 1985). γ-GT and 5-nucleotidase increased from week 8, while anaemia, elevation of sorbitol dehydrogenase (SDH), and serum glutamate oxaloacetate transferase (S-GOT) were reported in natural *F. gigantica* sheep infections by Haroun, Gadir and Gameel (1986) and in buffaloes (Swarup, Pachauri, Sharma and Bandhopadhyay 1987). In a phamacokinetic test Gatier, Coulet, Jean-Francois, Biro-Sauveur and Alvinerie (1994) reported that serum GLDH activities substantally increased in week four of infection while serum γ-GT activities increased eight weeks after infection

Wamae, (1996) reported that *F. gigantica* infected Friesians serum GLDH activities first increase 4 weeks after infection, *F. gigantica* infected Borans enzyme activities increased in the fifth week post infection. Peak activities of the enzymes occurred at weeks 13 and 14 weeks p.i. in the infected Friesians and Borans respectively. The activities of serum γ-GT increased from the twelfth week of infection in both infected groups reaching peak activities at week 16 p.i

Sheep infected with both *F. hepatica* and *F. gigantica* showed a significant increase in serum GLDH activities as early as two weeks after infection, by 3-5 wpi. these activities reached the peak. Although there was a transient increase by 3-5 weeks p.i. in serum γ-GT activities in these sheep a pronounced increase was first noticed in 8 weeks of infection. *F. gigantica* infected goats differed in that GLDH first increased in the fourth week of infection while γ-GT activities rose in the eleventh week after infection (Mahato, 1993)

2.8.1 Immunodiagnostic Techniques

The current conventional diagnostic methods are only effective after patency, when the flukes are in bile ducts and laying eggs (Taylor, 1964; Sewell, 1966; Bürger, 1992), even then the efficiency of egg detection assay is very low. The potential sensitivity and use in early helminths infections of immunodiagnostic methods is undoubtedly very high, but generally lack of specificity of immunoassays means that although they can be used in experimental infections they are of no value under field conditions (Kagan, 1979; Fagbemi and Obarisiagboni, 1991; Rodriguez-Perez and Hillyer, 1995).

Immunodiagnostic methods have potential because these methods may also find use detecting infection before patency therefore be able to assess the efficiency of therapeutic or control measures before and after patency. Thus increasingly epidemiological studies employ serological methods (Gaasenbeek, Over, Noorman and De Leeuw, 1992) in addition to the monitoring of experimental infection.

Anti-parasite antibody detection in serum and faeces

Copro-antibodies were compared in *Nippostrongylus braziliensis* infected rats following a primary infection (Wedrychowicz, MacLean and Holmes, 1983) and a 7-fold, 3-fold and about a 50-fold increase was measured of IgA, IgG and IgM respectively. Haemagglutinating antibodies appeared in the faeces on the third day after infection and were detected using E/S and worm somatic extracts as antigen in the assay.

El-Bahi Malone, Todd and Schorr (1992) using western blot techniques detected antigens in the faeces of cattle experimentally infected with *F. hepatica*.

Wedrychowicz, Turner, Pfister, Holmes and Armour (1984) reported a high IgG_1 and IgG_2 in serum and IgM and IgA in bile from sheep with primary *F. hepatica* infection but only slight increase in faecal IgA levels. Challenge infection dramatically elevated IgA levels in the faeces. Youssef, Mansour and Aziz (1991) in Egypt reported that Counterimmunoelectrophoresis (CIEP) was able to detect copro-antigens in early as well as chronic human fasciolosis

Parasite antigen detection in serum and faeces

An alternative method of diagnosis in parasite specific antibody detection assay is the detection of circulating antigens. One advantage of this method is that the assay can be designed to detect antigens produced by live parasites so that their detection means presence of active infection. Excretory Products or immune complexes in the tissues or excreta of the host suggests recent or active infection, thus overcoming the problem of tests based on antibody detection which can not distinguish between previous, current or re-infection. This method is thus potentially superior to and more accurate than the detection of anti-parasite antibodies since these remain in circulation some time after death of the parasite. Such a method was developed for diagnosis of *Taenia saginata* cysticercosis in cattle by use of a double antibody ELISA (Harrison, Joshua, Wright and Parkhouse, 1989).

Very few studies have been done on the detection of circulating antigens. Fagbemi, Obarisiagbon and Mbuh (1995), detected circulating antigens in *F. gigantica* experimentally or naturally infected cattle using antibodies against a specific 88-kDa antigen of *F. gigantica* by a double antibody ELISA. They were able to detect circulating antigens as early as the second and third week of infection. However, this procedure has the apparent advantage over the antibody detection in that it indicates current active infection, (Langley and Hillyer, 1989).

Using polyclonal sera in an antigen capture ELISA, Langley and Hillyer (1989) detected antigens in the serum of *F. hepatica* infected rabbits by 6-8 weeks post infection. The assay exhibited a better sensitivity than an antibody detection

ELISA but specificity and cross-reactions were not studied. It is postulated that sensitivity and specificity could be improved by the use of monoclonal antibodies.

Ellis, Gregory, Turnor, Kalkhoven and Wroth (1993) successfully developed an antigen capture ELISA to identify *Haemonchus contortus* antigen in faeces in naturally infected sheep.

Molecular biology

Recent advances in biotechnology have already been incorporated in the diagnosis of fasciolosis. There are some situations where the difficulty to identify differences between *Fasciola spp.* by morphological means has caused problems (Taylor, 1964). Even in *F. hepatica* and *F. gigantica* it is not possible to decide from the general body size to speciate some samples. In such situations, DNA probes offer advantages. The major advantage of this methodology is that it potentially permits detection of inter- and intra- specific genetic differences (Miller, 1990). In fact, recombinant DNA technique probably represents the most powerful method of diagnosis for many veterinary diseases.

Mahato (1993), when attempting to differentiate between *F. hepatica* and *F. gigantica*, developed four DNA probes. Two of these are species-specific and two are cross-reactive. The MHFh probe is specific *to F. hepatica* and the MHFg probe is specific for *F. gigantica*. Thus if used in conjunction, probes can differentiate between *F. hepatica* and *F. gigantica*.

The advent of the polymerase chain reaction (PCR) has allowed the amplification of very small amounts of parasite DNA (such as a single parasite egg) facilitating its identification (Miller, 1990; Harrison, 1991).

Marin, Prioto, Martin, Casais, Boga and Parra (1992) identified and expressed an *F. hepatica* gene encoding a gut antigen protein, 2fas1, bearing repetitive sequences. This antigen when used diagnostically was able to detect five weeks post infection.

The use of DNA probes in the epidemiological study of diseases has had wider usage in protozoal diseases (trypanosomosis, theileriosis, leishmanosis and malaria). This is because some protozoal species are morphologically similar but differ in their vectors and definitive hosts, pathology in infected hosts and their sensitivity to drugs. Identification of such organisms would help in the prediction of potential outbreaks of a particular disease and allow formulation of suitable control measures as well as evaluation of control measures (Majiwa, 1989). The advantages of DNA probes include their potential sensitivity. The selection of repetitive DNA sequence probes may help meet this requirement (Majiwa, 1989). Differentiation of *Fasciola spp.* especially in areas where both occur and the occurrence of intermediate forms has led to the utilisation of DNA mapping in attempts to aid speciation.

Blair and McManus (1989) distinguished between the two *Fasciola* species by restriction enzyme mapping of ribosomal DNA and found that each had two unique recognition sites.

2.9 VACCINATION AGAINST FASCIOLOSIS

There have been many of attempts to immunise animals against *Fasciola spp.* with crude antigen either in form of irradiated parasites (Boray, 1967), excretory/secretory (Burden and Hammet, 1980), surface or somatic antigens (Hughes, 1987) but generally there has been little progress made towards successful vaccinations against animal helminth parasites in contrast to those against bacterial and viral infections of veterinary importance.

The most promising vaccination studies to involve manipulation of a parasite defence enzyme have been with schistosome glutathione s-transferase (GST). The use of natural or recombinant GST candidate vaccine formulation to protect against schistosomiasis was reviewed by Emery (1996). The response to GST is directed to juvenile flukes reducing mean worm burdens by 78%. *Schistosoma mansoni* 28 kDa Glutathione S-Transferase (Sm28GST) is recognised as a potential anti-morbidity vaccine in human schistosomiasis as well as a diagnostic reagent in antigen detection. There is also extension of GST vaccination studies to *S. haematobium* in primates and S. bovis in ruminants (Hagen and Gryseels 1994). Another vaccine candidate is a high molecular weight (480 kDa) glycosylated antigen of *S. mansoni* found in soluble form in extracts of adult worms and eggs (Sm480); this proteolytic enzyme is reported to induce protective immunity (22-30%) in rats (Curtis, Fallon and Deonhoff, 1996). GST has also been used to vaccinate against *F. hepatica* infection in sheep. The enzyme gives significant protection against *F. hepatica* in sheep (mean reduction of 57% worm burden compared to control). However there are reports of poor protection with this enzyme (Brophy and Pritchard, 1992).

Sexton, Milner, Panaccio, Weddington, Wijffels, Chandler; Thompson, Wilson, Spithill, Mitchell, and Campbell (1990) showed that adult *F. hepatica* glutathion s-transferase (GST) can significantly protect sheep against liver fluke infection.

2.10 IMMUNOLOGY OF FASCIOLOSIS

2.10.1 Host species differences in the response to fasciolosis.

The sheep and goats and murine rodents are most susceptible to *Fasciola* spp. infection. Although they react severely to *Fasciola* spp, the immune response is insufficient to damage or isolate the parasite. In less susceptible animals cattle, humans, horses and guinea pigs, the response is usually to the late migratory stages and adult flukes and involves fibrotic tissue reaction and calcification. The other group of less susceptible animals are pigs, hamsters and carnivores. The tissue reaction happens much earlier which kills most of the flukes (Taylor, 1964; Barriga, 1981; Tizard, 1992). Thus the immune response varies from species to species.

In mice and humans, helminth infections are generally characterised by higher levels of IgE and eosinophils, induced by interleukin-4 (IL4) and interleukin-5 (IL5). These cytokines are produced by the helper T_2 (Th_2) subset of $CD4^+$ T cells (Finkelman, Pearce, Urban Jr and Sher, 1991).

2.10.2 Humoral Immune Responses to Fasciolosis

Duffus and Frank (1981) reported IgG_1, IgG_2 and IgM responses against the outer glycolyx antigen of juvenile flukes in cattle 2 weeks post infection and that IgG_1 was the dominant antibody isotype.

Analysis of parasite-specific immunogloblin isotypes IgM, IgG_1, IgG_2 and IgA by Clery, Togerson and Mulcahy (1996) showed IgG_1 to be the dominant isotype in both chronically infected cattle and previously naive heifers. There is clearly an involvement of humoral immunity even in the killing of *Fasciola* (Eckblad, Woodard and Lang, 1981; Pfister, 1984).

Hanna (1980) studied antigenic changes to the production of antibodies against T_0, T_1 and T_2 antibodies. He noted that the antibodies against T_0 and T_1 appear before 6 weeks post infection and T_2 is associated with the second antibody which appears after 6 weeks post infection. Hughes, Hanna and Symonds (1981) found both IgG and IgA in bile and sera of *F. hepatica* infected calves. The IgA was lower in serum and anti- T_2 antibodies were absent in serum and bile. Ogunrinade (1982) in agreement with Nansen (1974) found that *F. gigantica* infected cattle recorded a high IgG_1 and low of IgG_2, IgM and IgA isotype to E/S antigen. Burden, Hughes and Hammet (1982) found that all immunoglobulins are involved in humoral immune response in *F. hepatica* infected cattle but IgA and IgE might have a lesser influence. Pfister (1984) suggested that antibody IgE is involved in *Fasciola spp.* expulsion.

2.10.3 Cellular Immune Responses to Fasciolosis

A number of workers have reported on the possible damage to helminths by involvement of various types of leukocytes particularly eosinophils (Hughes, Anderson and Harness, 1976; Goose, 1978; Davis and Goose, 1981). Doy, Hughes and Harness (1978) suggested that the eosinophilia which develops in the intestinal

wall shortly after oral challenge with *F. hepatica* may be implicated in the rats' resistance to reinfection. Encapsulation by host cells, including eosinophils has been demonstrated when newly excysted flukes are injected in the peritoneal cavity of sensitised rats and Davies and Goose (1981) confirmed that peritoneal eosinophils play a vital role in killing of flukes.

Eosinophilia in cattle and sheep infected with *Fasciola spp.* appears at least two to four weeks post infection and after subsiding fluctuates slightly above the normal level. Several reports on the steps leading to the killing of helminths by eosinophils indicate that first the eosinophils adhere to the surface of the parasite then degranulate, releasing peroxidase directly onto the parasite or first in the cytoplasmic vacuole and it is this enzyme that causes damage.

Doy and Hughes (1982) concluded that there are two different mechanisms capable of inducing an eosinophilia in rats. One of these mechanisms is T-cells dependant and the other T-cells-independent depending upon the stimulus, in their case (the parasite). Oldham and Williams (1985) demonstrated the presence of antigen sensitive T-cells (ASTC) in the peripheral blood of *F. hepatica* infected calves by lymphocytes proliferation and Interleukin-2 (IL-2) production tests. Zhu, Lukas and Boros (1994) found after studying lymphocytes from liver granuloma of *S. mansoni* infected mice that Th_0- and Th_2-type granuloma lymphocytes play a role in parasite egg-induced granuloma formation.

One of the first results to describe the cytokines response of specific T cells obtained from 36 and 40 weeks after *F. hepatica* infected cattle by Brown, Davis,

Dobbelaere, and Rice-Ficht (1994) found that most T-Cell clones were Th_2 (strong IL4) as well as less restricted, IL2, IL4 and Interferon gamma (IFNγ) or IL2 and IL4 or IL4 and IFNγ, TH_0 and very little or no Th_1 (IL2) cytokines profiles. After lymphocyte proliferation there was no production of IFNγ by lymphocytes responding to adult fluke antigen suggesting strongly therefore that the response by these two animal groups was not a TH_1 type response but that of a Th_2 or Th_0 cell type (Clery, Togerson. and Mulcahy, 1996).

2.10.4 Mechanisms of Immunity

Glycocalyx turnover or replacement in newly excysted juvenile (NEJ) *F. hepatica* flukes has been suggested by Hanna (1980) as a possible mechanism for protection against the host immune responses. During incubation in the presence of immune serum this glycocalyx is continuously shed and replaced, and it has been postulated that the lack of *in vitro* killing of *F. hepatica* NEJ's is due, in part, to the rapid turnover of this highly antigenic layer (Duffus and Franks, 1981) preventing the attachment of both antibodies and/or cells.

Traditionally it has been accepted that immunity to helminths involves eosinophils, mast cells and IgE, and it is reasonable to assume that host immunity against helminths might be dependant on Th_2 cells (Finkelman, Pearce, Urban, and Sher, 1991). Following infection with parasitic helminths, a clonal expansion of antigen-specific T-cells occurs, and the subsequent and concerted immune response is regulated by cytokines secreted differentially by subsets of helper T-cells, currently designated TH_1 and TH_2 cells. A good example would be the most

thoroughly studied nematode *Heligmosomoid polygarus* in the mouse where it would appear that immunity is mediated by a TH_2-cell population through the production of Interleukine 4 (IL-4) (Brophy and Pritchard, 1992)

The excretory and secretory products of helminths exert a wide range of influences on the immune response of their mammalian host, from the induction of host-protective immunity to providing mechanisms by which the parasites evade the host immune response. Different immunosuppressive roles have been reported by several workers. Goose (1978) suggested that *F. hepatica* E/S products were toxic to rat leunphocytes and that these substances might protect the parasite from it's host immune defence. Zimmerman, Kerkvliet, Braun and Carro (1983) reported an immunesuppression of lymphoproliferation in sheep infected with *F. hepatica* and suggested that this may explain the failure of sheep to develop resistance to *Fasciola spp.* infection. Vieira, Gazzinelli, Kussel, De-Sousa and Colley (1986) demonstrated that *S. mansoni* E/S products were able to decrease the proliferative response by mice and human lymphocytes. In contrast Lightowlers and Richard (1988) observed a stimulation of the lymphoproliferation in *S. mansoni*, as also Poitou, Baez and Boulard (1992) reported for *F. hepatica*.

CHAPTER THREE

MATERIALS AND METHODS

3.1 SOURCES OF METACERCARIAE

3.1.1 Commercial Source of *F. hepatica* (British strain) Metacercariae

The *F. hepatica* metacercariae used to infect the three sheep in experiment 1, and in experiment 3 were purchased from the Compton Paddock Laboratories, Newbury, Berks, England.

3.1.2 *Fasciola gigantica* (Kenyan strain) Metacercariae Source

The *F. gigantica* metacercariae used in experiment 4 were obtained from the National Veterinary Research Centre, Muguga, Kenya. The *F gigantica* and those used in experiment 6 were obtained from the Centre for Tropical Veterinary Medicine (CTVM) laboratory Kenya isolate of the parasite (See section 2.1.3).

3.1.3 Laboratory Maintenance and Supply of *Fasciola hepatica* (Peruvian strain) Metacercariae

F. hepatica (Peruvian strain) metacercariae for sheep 9 and 10 of experiment 1 and experiment 5 were produced at CTVM Edinburgh by Mr. H.R. Urquhart. These metacercariae were obtained from experimentally infected *L. viatrix.*

Culture of green algae

The techniques used for culturing the green algae were those described by Taylor and Mozley (1948) with some modifications. Top soil with as few stones as

possible was collected. The soil was then passed through a 4 mm mesh sieve to remove remaining stones and other debris. This fine soil was then autoclaved at 1 kg/ cm^2 for 30 minutes. After cooling, the soil was mixed with 500 ml of mineral solutions (See Appendix 1.) as described by Sewell (1973).

This mixture was smoothly spread at the bottom of 104 mm by 178 mm plastic boxes (Stewart Plastic Products Ltd) to 15 mm in depth. The algae from a previously prepared feeder culture were then transplanted into the middle of the box. After gently spraying the soil with distilled water the boxes with algae were placed in a warm room at 23^0C, about 60 mm beneath a 38 Watt white fluorescent light bulb (Gro-Lux, Sylvania, Germany). The algal cultures were gently sprayed with distilled water every day.

Production of young snails

Egg masses from adult *L. viatrix* were placed onto a fresh algal culture and maintained at 23^0C to hatch. After hatching and eventual depletion of the algae, the young snails were carefully collected using tissue forceps and transferred onto a fresh algal culture.

Production of miracidia

F. hepatica eggs were imported from Peru. These eggs were put into 50 ml flasks (Nunclon, Gibco code 1-63371A) and incubated for five days at 23^oC. During this initial incubation the eggs were washed daily with distilled water. The flasks were then wrapt in aluminium foil and kept in the dark for 9 days at 23^oC.

Miracidiae hatching was achieved by decanting the embryonated the eggs into a petri-dish and exposing them to light at room temperature.

Infecting snails with miracidia

Infection of *L. viatrix* was carried out as described by Haroun (1979) for *L. truncatula*. The snails were infected when they were 2 to 5 mm in length. Seven to ten miracidia were pipetted into each well of a flat-bottomed 96 (317µl each) wells polystyrene plate (Gibco, code 2-62162A). The snails were placed individually into each of these wells and the plates covered and left for 4 hours at room temperature, after which the infected snails were transferred onto a fresh algal culture. The infected snails were maintained at 23°C and transferred to fresh algal plates as required. Five weeks after infection the snails were examined under a stereomicroscope to determine if they contained *F. hepatica* rediae. Snails that did not contain rediae were separated and re-examined after two weeks and those showing no evidence of infection at that time were kept as egg-laying adults.

Harvesting metacercariae

Snails harbouring mature (6-week or more) *F. hepatica* infections were placed in small (120 x 75 mm) polythene bags containing 40 ml of cold (4°C) distilled water. The polythene bag was placed in a bottle and left at room temperature. The cercariae were shed as the water warmed to room temperature. The snails were removed from the water after approximately 4 hours and placed onto a new agal plate. The bag containing the metacercariae was left at room temperature for 3 days, after which the water was decanted, leaving a few drops in the bag. The

bag was kept in the refrigerator at 4°C for a few days until needed. The discarded water was autoclaved in order to kill any remaining metacercariae.

Precaution in handling Fasciola spp

Both *F. hepatica* and *F. gigantica* infect a wide range of animals including man. The only stage infective to man is the metacercariae, and infection is by ingestion.

According to the Scottish Advisory Committee on Dangerous Pathogens *Fasciola* spp are level 2 containment pathogens and special guidelines exist for their safe handling. Procedure involving infective material was carried out in accordance with the code of practice for handling *Fasciola* spp. material in designated snail rooms. Infected animals were handled in accordance with the regulations of the Animal (Scientific Procedure) act of 1986 and specifically in accordance with the protocols laid down in Project Licences PPL60/01582 and PPL60/1583.

Gloves were worn when handling metacercariae. The metacercariae were removed from storage in the snail room only for the purpose of infecting animals. During storage and transportation to the animal house the infective material was contained in non-breakable screw topped containers. All such vessels were suitably marked with biohazard tape. All materials used in this process were new and equipment was sterilised before reuse.

3.2 EXPERIMENTAL ANIMALS AND DESIGN

The experiment groups 1 and 2 monitoring was conducted by Mahato (1994) in a pathogenesis experiment. Experiment 6 monitoring was conducted by M.

Nyanzunda (1993). Sampling started 2 weeks prior to and continued weekly up to 22 weeks after the day of infection

3.2.1 Experiment 1: *F. hepatica* (British and Peruvian strains) Infection in Sheep

Six sheep, four female Scottish Blackface sheep of about 11 months old, one 18 months old female Scottish Blackface sheep and one 18 months old Suffolk cross sheep were purchased from the local farms within the Veterinary field Stations large animal practitioners territorial unit. The sheep were injected with a single dose of a 1.0% weight per volume (W/V) Ivermectin (IVOMEC®. MSD AGVET Ltd.) to eliminate intestinal nematodes.

The animals of this group were divided into three subgroups. As described in the Table 3.1, five animals were infected with *F. hepatica* metacercariae. The remaining one was left uninfected. Two of the animals from this group were infected with 200 and the other three with 300 metacercariae of *F. hepatica* and one sheep in this group was left trematode free and used as a control.

Table 3.1: Experiment 1: Experimental details of chronic *F. hepatica* (British and Peruvian) strains infection in sheep

Experiment 2	Animal No.	Breed	Sex	Metacercariae given (No)	Fluke recovery (No)	(%)
Higher dose	5	Blackface	F	300	75	33
	6	Blackface	F	300	150	50
	7	Blackface	F	300	172	73
Lower dose	9	Blackface	F	200	-	-
	10	Suffolk S	F	200	-	-
Uninfected controls	8	Blackface	F	0	0	0

3.2.2 Experiment 2: *F. hepatica* (British strain) Infection in Sheep

Six sheep, 2 male and 1 female Scottish Blackface sheep, and 1 male and 2 female Suffolk crosses, all approximately 18 months old and raised under fluke-free conditions, were obtained from the Firth Mains Farm of Moredum Research Institute, Edinburgh. The sheep were orally treated with a single dose of 5 mg/kg body weight oxfendazole (Systamex, Wellcome Foundation Ltd., London) to eliminate intestinal nematodes.

The animals of this group were divided into three pairs. As described in the table 3.2, animals allocated in two of the three pairs were infected with *F. hepatica* metacercariae. The remaining group was left uninfected. One pair of animals from this group was infected with 150 and the other with 350 metacercariae of *F. hepatica* and the other pair of sheep in this group were trematode free animals and used as controls.

Table 3.2: Experiment 2: Experimental details of chronic *F. hepatica* (British strain) infection in sheep

Experiment 2	Animal No.	Breed	Sex	Metacercariae given (No)	Fluke recovery (No)	(%)
Lower dose	24	Suffolk X	F	150	77	51.3
	28	Suffolk X	M	150	95	63.3
Higher dose	26	Blackface	F	350	247	70.6
	30	Blackface	M	350	203	58.0
Uninfected	22	Suffolk S	M	0	0	00
controls	32	Blackface	F	0	0	0

3.2.3 Experiment 3: *F. gigantica* (Kenyan strain) Infection in Sheep

Nine sheep, all female Scottish Blackface, approximately 2 years old and were purchased from the local farms within the Veterinary Field Station's large animal practitioners territorial unit. The sheep were orally treated with a single dose

of 1.0% w/v Ivermectin (IVOMEC*. MSD AGVET) subcutaneously to eliminate intestinal nematodes. Although there were no fluke eggs in the initial faecal egg count examination all the sheep were drenched with 5% w/v triclabendazole (Fasinex® 5%. CIBA GEIGY) at a rate of 8 ml per 40 kg of sheep.

The animals of this sub-group were divided into two groups. As described in Table 3.3, animals allocated in sub-group one were infected with 100 *F. gigantica* metacercariae each and the sheep in the other group were trematode free animals and used as controls.

Table 3.3: Experiment 3: Experimental detail of chronic *F. gigantica* (Kenyan strain) infection in Scottish Blackface female sheep

Experiment 4	Animal No.	Live weight	Metacercariae given (No)	Fluke recovery (No)	(%)
Infected	11	51	100	34	34
	12	39	100	50	50
	13	40	100	45	45
	14	41	100	58	58
	15	42	100	76	76
Uninfected	16	40	0	0	0
controls	17	41	0	0	0
	18	43	0	0	0
	19	47	0	0	0

3.2.4 Experiment 4*: F. gigantica* (Kenyan strain) Infection in Sheep

Six sheep, 2 male and 1 female Scottish Blackface sheep, and 1 male and 2 female Suffolk cross sheep, all approximately 18 months old and raised under fluke-free conditions, were obtained from the Firth Mains Farm of Moredum Research Institute, Edinburgh. The sheep were orally treated with a single dose of 5 mg/kg body weight Oxfendazole (Systamex, Wellcome Foundation Ltd., London) to eliminate intestinal nematodes.

The animals of this group were divided into three pairs. As described in the table 3.4, animals allocated in two of the three pairs were infected with *F. gigantica* metacercariae. The remaining group was left uninfected. One pair of animals from this group was infected with 150 and the other with 350 metacercariae of *F. gigantica* and the other pair of sheep in this group were trematode free animals and used as controls. Details of each animal including fluke recoveries are shown in Table 3.4.

Table 3.4: Experiment 4: Experimental details of chronic *F. gigantica* (Kenyan strain) infected sheep

Experiment 4	Animal No.	Breed	Sex	Metacercariae given (No)	Fluke recovery (No)	(%)
Lower dose	23	Suffolk X	F	150	80	53.3
	27	Suffolk X	M	150	83	55.3
Higher dose	25	Blackface	F	350	181	51.7
	29	Blackface	M	350	34	9.7
Uninfected	21	Suffolk X	F	0	0	0
Controls	31	Blackface	M	0	0	0

3.2.5 Experiment 5: Chronic *F. hepatica* (Peruvian strain) Infection in Calves

Six calves, 5 male and 1 female, all Friesian between 10 to 12 weeks old and raised under fluke-free conditions, were obtained from the Bush Farm, University of Edinburgh..

As shown in the Table 3.5, 5 calves were infected with *F. hepatica* metacercariae. One calf from this group was uninfected. Sampling started 2 weeks prior to and continued weekly up to 27 weeks after the day of infection

Table 3.5: Experimental detail of chronic *F. hepatica* (Peruvian strain) infection in Friesian calves (wppi = Weeks post primary infection)

Experiment 5	Calf No./Sex	Body weight (Kg)	Primary infection metacercariae given	Challenge infection metacercariae given/wppi
Single infection	14c/F	82	200	
Infection/challenge	15c/m	91	200	100/15
Infection/challenge	23c/m	108	600	100/25
Single infection	34c/m	71	450	
Single infection	45c/m	69	450	
Uninfected control	26c/m	85	0	

3.2.6 Experiment 6: Chronic *F. gigantica* (Kenyan strain) Infection in Calves

Four male Friesian calves of about 12 weeks of age were bought from Bush Farm, University of Edinburgh. There were no helminths eggs at the initial faecal egg count examination. Three calves (calves 22,23 and 24) were infected and one (calf 26) was kept as uninfected control. The animals were monitored as an ongoing research program at CTVM by Nyanzunda (1993).

As shown in the Table 3.6 below, 3 calves were infected with 400 *F. gigantica* metacercariae, however weights were not taken. Sampling started 2 weeks prior to and continued weekly up to 33 weeks after the day of infection

Table 3.6: Experiment 6: Experimental detail of chronic *F. gigantica* (Kenyan strain) infection in Friesian male calves.

Experiment 6	Animal No.	Metacercariae given (No)	Fluke recovery Fluke No.	%
Infected	calf 22	400	30	7.5
Infected	calf 23	400	31	7.75
Infected	calf 24	400	10	2.5
Uninfected	calf 26	0	0	0

3.3 MAINTENANCE OF EXPERIMENTAL ANIMALS

Sheep were housed in concrete pens and provided with wood shavings as bedding. They were fed a daily ration of 300g cubes Sheep Food® (dry matter 88%; protein 18%, 0_2l 2.5%, Fibre 10% and ash 8% of the dry matter) per head. Water and hay were provided *ad libitum*. Calves were housed in concrete pens and provided with straw for bedding. They were fed *ad libitum* a hay diet. The water was provided *ad libitum*.

3.4 INFECTION OF EXPERIMENTAL ANIMALS

The polythene bag containing metacercariae was opened with scissors and cut into manageable pieces. The metacercariae were examined for viability under a stereomicroscope at magnification of 50X. Those metacercariae having a characteristic granular appearance were considered as viable (Hammond, 1970).

The required number of metacercariae for experiments 1, 2 and 6 were counted and then scraped and washed off the polythene into a plastic petri dish using a microscope glass slide as scraper. The water, containing metacercariae, was poured onto a Whatman No. 4 filter paper in a glass funnel making sure that no metacercariae were left in the petri dish. After the paper had drained, it was folded, with the retained metacercariae inside, and administered orally to the animals using a balling gun.

For experiments 3, 4 and 5, the required number of metacercariae were counted under a stereo microscope while still on the polythene. The polythene was cut into thin strips which were laid on a sheet of Whatman No. 4 filter paper. The

filter paper was folded into a ball with the polythene containing the metacercariae to the inside. The filter paper ball was then orally administered using the balling gun.

3.5 MONITORING OF ANIMAL EXPERIMENTS

The experiments three and four was monitored by Mahato (1993) as part of his PhD. research work. The Cattle in experiment six were monitored by the staff in helminthology laboratory and Nyanzunda (1993) as part of his MSc. project.

3.5.1 Measurements of Weights

A weighing crush (Weighbridge, Leslie P. Morris Lt, Salop) was used to weigh the sheep in the experiments at CTVM. The calves were not weighed.

3.5.2 Sample Collection, Preparation and Storage

Blood and serum sample collection

The blood samples for haematological studies were taken from the jugular vein into vacutainers containing disodium or dipotassium ethylenediamine tetra-acetic acid (EDTA) as an anticoagulant. The blood samples for biochemistry and serology were obtained from the jugular vein into plain vacutainers (Becton, Dickinson and Co Ltd, UK) and allowed to clot in an incubator at 37^0C for 1 hour, then kept at 4^0C overnight for the clot to retract. The serum was separated by centrifugation at 2,000g for 30 minutes, dispensed into 2 ml aliquots and stored in labelled eppendorf tubes frozen at -20^0C until required.

Faecal samples collection

About 3 grams of faecal material was used for coproscopic assays and two grams to prepare supernatant for immunodiagnostic assays. The two grams for immunodiagnostic assays was thoroughly homogenised and mixed with 3 ml of sample Phosphatase Buffered Saline (PBS) pH 7.4 containing 0.02% v/v sodium azide and 0.05% w/v Tween 20®. The suspension was then mixed and centrifuged at 2,000g at 4^0C for an hour. The supernatant was collected in 2 ml vials and stored at -20^0C until use. In experiment 3 whole faecal samples were also collected and stored in 4 ml vials at -20^0C.

3.5.3 Faecal Egg Counts

A differential centrifugal flotation technique as described by Hammond and Sewell (1972) was used to calculate the fluke egg counts. Faecal samples in zinc sulphate solution were centrifuged until slight convex meniscus was formed. A cover slip was placed over the meniscus, left to stand for about 3 minutes, and then lifted off vertically, placed on a microscope slide and examined for the presence of *Fasciola* spp. eggs.

Nematode eggs counts were estimated according to the method described in the Technical Bulletin No. 18 (Anon, 1977) with minor modification. A 3g sample of faeces was weighed and homogenised in 42 ml. of water. A 15 ml. centrifuge tube was filled and centrifuged for 5 minutes at 1500 g. The supernatant was decanted and the sediment resuspended in saturated sodium chloride to the 15 ml mark. After thorough mixing two chambers of a McMaster slide were filled and eggs counted in

both chambers. The total number of eggs counted was multiplied by the factor of 50 and results expressed in egg counts per gram of faeces (EPG).

3.5.4 Haematological Techniques

Total erythrocytes and leukocyte counts

These were determined by the method described in the Reference Manual for the Coulter Counter Model ZM (Coulter Electronics, 1985), using an electronic cell counter (Coulter Counter Model Z, Coulter Electronics Ltd, Luton). The dilution used for the total leukocyte count was 1:500 and 1:25,000 for erythrocyte counts. The red blood cells (erythrocytes) were expressed in real numbers $x10^{12}$ and the white blood cells (leukocyte) were expressed in real numbers $x10^{9}$.

Haemoglobin estimation

Haemoglobin, converted to cyanmethaemoglobin with Zapoglobin (Coulter Electronics Ltd, Luton) was measured in a Haemoglobinometer (Coulter Electronics Ltd, Luton). The haemoglobin concentrations were expressed as grams per decilitre (g/dl) (Archer, 1963).

Packed cell volume determination

Packed cell volume (PCV) was determined using a Hawksley micro-haematocrit centrifuge and reader (Hawksley and Sons Ltd, Lancing, England). The samples were centrifuged at 12000g for 7 minutes and the volumes were expressed as percentage.

Eosinophil counts

These were done by a modification of the method of Archer (1963) using a modified Fuschs Rosenthal Chamber (Weber Scientific International Ltd, Lancing, England). EDTA blood 20µl was added to 0.38 ml of Discombe's fluid (10ml eosin (200g/l), 10ml acetone and 80ml distilled water) to give a 1 to 20 dilution. This was mixed for not longer than 30 seconds before filling both Fuchs-Rosenthal counting chambers. The eosinophils were counted in all ruled areas in the chambers.

The total volume in the chamber is 3.2µl. The above information provided a formula to calculate the eosinophils counts per µl. The eosinophil count results were expressed in 10^9/l.

Calculation: If N eosinophils are counted in 3.2µl then the total eosinophil count per ml is equal to (=) $\frac{N \times 20(\text{dilution})}{3.2} \times 10^6$/litre = N × 6.25 × 10^6/litre. eosinophils counted were expressed in real numbers x10^9.

Differential leukocytes counts

Thin blood smears were made on clean microscope glass slides using EDTA blood (Chance Propper Ltd, England) and air-dried. The slides were fixed for two minutes in methanol and then stained for 30 minutes in Giemsa's (British Drug House Ltd. (BDH) stain diluted 1:10 with distilled water. The slides were washed with Giemsa's buffer (BDH) pH 7.2 and allowed to air-dry. Differential leukocyte counts were performed on the stained slides by differentiating a total of 200 cells in successive microscope fields using the x 1000 magnification on a Leitz Dialux 22 microscope (Leica UK). The proportions of different white blood cells were

calculated as a percentage and these were converted to real numbers. Results were expressed as real numbers $x10^9$.

3.5.5 Biochemical Techniques Conducted on Serum Samples

Total protein

Total serum protein was determined by the Biuret method as described in total protein procedure Anon (1994), using Randox total protein reagent and protein standard. The procedure is based on the principle that the copper ions in alkaline Biuret reagent, react with the peptide bonds of serum proteins to form a purple colour with an absorbance maximum at 540 nm. The intensity of colour is proportional to the total protein concentration which was measured in optical density using a spectrophotometer (PU 8600 UV/VIS, Pye Unicam Ltd, Cambridge). The reliability of test results were monitored by routine use of control sera. Calculation: Since a standard was used the Total Protein Concentration in gram per decilitre (g/dl).

$$(g/dl) = \frac{A \ \ 540 \text{ nm (sample)}}{A \ \ 540 \text{ nm(standard)}} \times 6$$

Albumin

Serum albumin was estimated as described by Anon (1994). using Randox albumin reagent and an albumin standard. The measurements of serum albumin is based on its quantitative binding to the indicator 3,3',5,5'-tetrabrom-m cresol sulphonphthalein (bromcresol green) to produce a blue green colour with an absorbance maximum at 628 nm. The intensity of the colour produced is directly proportional to albumin concentration in the sample and was measured using the Pye Unicam PU 8600 UV.VIS spectrophotometer (Pye Unicam Ltd, Cambridge) at room

temperature. The albumin concentration, expressed as gram per decilitre, in the sample was calculated from the following formula: The results were expressed in gram per litre (g/l).

$$\text{Albumin concentration (g/dl)} = \frac{\text{A 628 nm (sample)}}{\textbf{A } 628 \text{ nm (standard)}} \times \text{Concentration of Standard}$$

Glutamate dehydrogenase (GLDH) estimation

The activity of GLDH (EC 1.4.1.3) in the serum was measured, without determination of non-specific creep reaction, using a kit, Test-Combination GLDH activated (Randox Ltd.). The activities of GLDH in the samples were expressed as Unit per litre (U/L).

The test is based on the following principal:

$$\alpha\text{- Oxoglutarate} + \text{NADH} + NH^{+}_{4} \xrightarrow{GLDH} \text{Glutamate} + \text{NAD} + H_20$$

Gamma glutamyltransferase (γ-GT) estimation

The activity of γ-GT (EC 2.3.2.2) in the serum was assayed using a kit, ι-GT (Randox) which is based on the following principal:

$$\text{L-g-Glutamyl-3-Carboxy-4-Nitroanilide} + \text{Glycylglycine} \xrightarrow{\gamma - GT} \text{Glutamylglycygllycine} + \text{p-Nitroaniline}$$

Spectrophotometric readings were taken at 25°C and 405nm. The calibrated standards. The activities of γ-GT in the samples were expressed as Unit per litre (U/L).

Glucose and b-Hydroxyl-butyrate analysis

The glucose and β-Hydroxylbutyrate were analysed using an automatic chemistry analysing machine (Opera Chemistry Systems, Bayer®, Leverkusen,

Germany). The glucose and β-Hydroxylbutyrate results were expressed in milimole per litre (mmol/l).

3.5.6 Pathological Techniques

Post mortem procedures

Post-mortem examinations were carried out on all animals soon after they died or were humanely killed. The method used for recovery, counting and measuring the flukes are describe by Nyanzunda (1993). A careful examination was made of all internal organs for abnormalities, with particular attention to their colour and appearance and any sign of ectopic flukes. The animal was eviscerated and the liver with intact bile duct was placed on a clean white 370 mm × 300 mm enamel pan and weighed. The bile ducts were cut open and the flukes in them collected using forceps. In order to make sure that most fluke have been collected and counted, the liver was then cut into small pieces of about 45 mm × 45 mm. The collected flukes were counted and immediately put in sterilised plastic container with sterile warm (37^0C) PBS (code BR14a) pH 7.3 (Oxoid Ltd. Basingstoke, Hampshire, England). Part of liver parenchyma, large bile ducts and hepatic lymph nodes lymph nodes were stored in 10% v/v Formalin for histology.

Histopathology

Samples of liver and other tissues were fixed in 10% v/v buffered formalin-saline. Histological slides stained with haematoxylin and eosin (H&E), Maritus scarlet blue (MSB), carbolchromotrope (CC) and Prussian Blue Reaction (PBR) were

prepared by staff at the Veterinary Field Station, Department of Pathology, Royal (Dick) School of Veterinary Studies.

3.6 PREPARATION OF *FASCIOLA* SPP. EXTRACTS

The flukes collected at *post-mortem* examination were washed, i.e. until they were free of any visible bile pigments, 6 times in warm (37°C), sterile PBS, then 6 times in sterile PBS containing penicillin (100 IU/ml), streptomycin (100μg/ml) and fungizone (2μg/ml) and washed further 6 times in RPMI medium containing penicillin (100IU/ml), streptomycin (100μg/ml) and fungizone (2μg/ml. Following these washes, the treatment of the flukes varied depending on the type of extract to be made. The adult fluke extracts prepared from both *F. hepatica* and *F. gigantica* are as described in the table 3.7, however only excretory secretory extracts were used in these studies.

Table 3.7 The adult Fluke Excretory-Secretory extracts prepared from both *F. hepatica* and *F. gigantica.*

Fasciola species	0-24 hours Culture	24-48 hours Culture
F. hepatica	+	+
F. gigantica	+	+

3.6.1 Adult Fluke Excretory and Secretory (E/S) Products

The washed flukes were placed into tissue culture flasks containing RPMI 1640 medium with penicillin (100 IU/ml), streptomycin (100μg/ml), fungizone (2μg/ml), sodium pyruvate and L glutamate at 1 fluke per 5 mls of medium. The flasks containing the flukes were incubated in a 6% (v/v) carbon dioxide in air incubator at 37°C for 24 hours. Following 24 hours maintainance, the medium was

collected and fresh medium added to the flasks. Medium collected from the 24 hours culture was centrifuged at 2000g, for 30 minutes, at 4°C, the supernatant collected and the protein content determined below, (see section 3.5.5). The same procedure was followed for the medium collected 24-48 hours incubation and the 2 solutions were designated 0-24 and 24-48 hours E/S respectively, aliquoted into 1ml volumes and stored at -70°C.

3.6.2 Source of Other Purified Antigens and Monoclonal Antibodies.

Cathepsin-L like cysteine protease, 27 kDa, from adult *F. hepatica* was supplied by Professor John P. Dalton of the School of Biological Sciences, Dublin City University, Ireland. This antigen was purified and characterised as described by Smith, Dowd, McGonigle, Keegan, Brennan, Trudgett and Dalton (1993). Adult *F. hepatica* Glutathione S-Transferase (FhGST) was supplied by Professor G. V. Hillyer of University of Puerto Rico, School of Medicine, Puerto Rico. The FhGST was purified directly from the parasite as described by Hillyer, Soler de Galanes and Battisti (1992)

The rat monoclonal antibodies used in sheep were provided by Dr. J. Hopkins of the department of Clinical studies, Royal (Dick) Vet. School, University of Edinburgh, Scotland.

3.6.3 Estimation of Protein Content

The protein concentration of the E/S products was estimated by the method described by Warburgh and Christian (1941).

This technique depends on the phenylalanine, triptophan and tylosin content in the sample and so does not necessarily measure total protein accurately. It is however particularly useful because it is easy and quick to use. The extinction of an appropriately diluted solution of sample was measured at both 260 nm and 280 nm in a 0.2 mm light-path quartz cells (© Unicam Ltd. Cambridge) in a Unicam 8625 Series spectrophotometer (© Unicam Ltd. Cambridge). The E260 : E280 ratio was calculated, using this ratio the proportion of nucleic acids in the protein sample and a factor for calculating the protein content was read off from the standard table (Warburgh and Christian, 1941). The protein concentration was calculated using the formula:

$$\text{Protein Concentration (mg/ml)} = \text{Extinction at 280 nm} \times \text{Factor} \times \frac{1}{d}$$

where d is length of light-path in centimetres.

3.7 INDIRECT ENZYME LINKED IMMUNOSORBENT ASSAYS (ELISA)

Two Enzyme Linked Immunosorbent Assay (ELISA) systems were employed for detection of parasite specific antibody in serum and faecal samples; the first using the polyclonal conjugate (total Ig and IgM) as described by Nyanzunda (1993) and the other detection incorporating immunogloblin subclasses using rat monoclonal antibodies to sheep IgG1, IgG2 and IgA. In the ELISA assays either 48 hours *F.hepetica* and *F. gigantica* excretory/secretary products (Fh-E/S or Fg-E/S), adult *F. hepatica* Cathepsin-L1 cysteine protease (Fh-Cathepsin) or adult *F. hepatica* Glutathione S-Transferase (Fh-GST) were employed as antigen.

3.7.1 Indirect ELISA Protocol

Polystyrene microtiter immunoassay plates (Dynatech laboratories, Inc. London, UK.) arranged in a 96-well, flat-bottomed format, were coated with 50 µl/well of *Fasciola.* spp extracts, diluted in borate buffered saline pH 8.2 (BBS, appendix 1). The plates then were covered with plastic film to prevent evaporation and left overnight at 4 °C. The contents of all the wells were then shaken out and the wells washed three times in a 0.9% (w/v) solution of sodium chloride containing 0.05% (v/v) Tween 20 (washing solution, appendix 1) allowing 3 minutes for each wash. The plates were then well drained. Normal serum of the same species as the conjugate, diluted to 4% (v/v) in PBS pH 7.3 (appendix 1) containing 0.05% (v/v) Tween 20 (blocking solution, appendix 1) was added, at 100 µl/well to all wells, the plates were incubated for 1 hour at room temperature and then the blocking buffer was shaken out and the plates blotted by tapping on absorbent paper. Positive and negative controls, all diluted in blocking solution, were added to appropriate wells at 50 µl/well. The plates were incubated for 1 hour at 37 °C. For every incubation step, the plates were covered with plastic film to prevent evaporation, then after incubation subjected to three washes of 3 minutes duration in washing solution. Anti-serum, conjugated to horse-radish peroxidase (HRP), and optimally diluted in blocking solution, was added at 50 µl/well to all. The plates were again incubated for 1 hour at 37 °C, washed, following which the peroxidase substrate, tetramethyl benzidine (TMB, Kirkegaard & Perry Laboratories, Gaithersberg, USA. lot SG24) was added to all wells. The reaction was allowed to develop for 10-30 minutes (making sure the background remained acceptably low) at room temperature and then

stopped by the addition of 50 µl/well of 0.2 Sulphuric Acid (appendix 1). The optical density (OD) was measured at 450 nm in an automated ELISA reader (Multiskan® version 2.03. Labsystems. Basingstoke Hants, UK). ELISA plate layout for the assay for sequential analysis is shown in Table 3.10.

3.7.2 The Indirect ELISA with Polyclonal Antibody Detection System.

This assay was used to monitor the total immunoglobulin response to the three antigens, E/S products, Fh-Cathepsin and Fh-GST and the IgM response. Blocking serum and indicator details are shown in Table 3.8. The polyclonal detection system followed a general protocol as detailed in Figure 3.1

Table 3.8 Reagent details for polyclonal antibody detection system in sheep and in cattle. The conjugates were purchased from three different companies, i.e. Bethyl Laboratories INC (Bethyl Lab. INC. USA), Kirkegaard and Perry Laboratories INC (Kirkegaard Lab INC. Gaithersberg, USA) and Nordic immunological Laboratories (Nordic Lab. Halland)

Sheep	Assay	Blocking	indicator	
	Total Ig	Normal rabbit serum	Rab. anti-sh. Ig (H+L) lot 3718	Nordic Lab
	IgM	Normal rabbit serum	Rab anti-sh IgM (μ) lot JJ 21-1	Kirkegaard Lab INC.,
Cattle				
	Total Ig	Normal goat serum	Gt. anti-B. Ig (H+L) lot 3716	Nordic Lab.
	IgG_1	Normal goat serum	Gt. anti-B IgG_1 (Heavy chain) Lot. A10-116p-4..	Bethyl Lab. INC
	IgM	Normal goat serum	Gt. anti-B IgM (μ) Lot.A10-101p-6	Bethyl Lab. INC
	IgG_2	Normal goat serum	Gt. anti-B IgG_2 (Heavy chain) Lot.A10-117p-6.	Bethyl Lab. INC
	IgA	Normal goat serum	Gt. anti-B IgA (Heavy chain) Lot.A10-121p-7	Bethyl Lab. INC

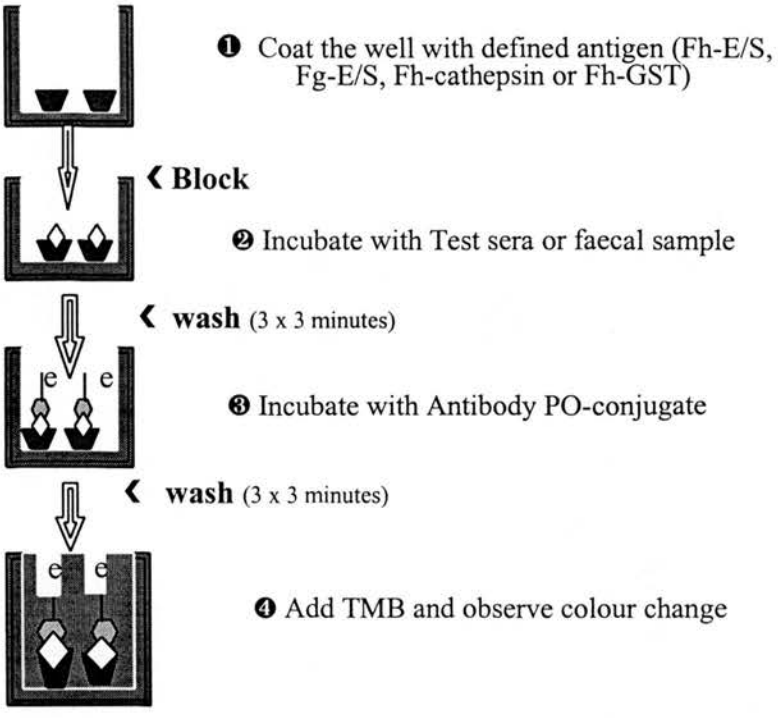


Figure 3.1 The indirect ELISA with polyclonal antibody detection system used in this study.

3.7.3 Indirect ELISA Protocol for Monoclonal Antibodies Detection System

The indirect ELISA was carried out by a modification of the general (polyclonal) procedure described in section 3.7.1. The difference is that one more step was incorporated between the test sera or faecal samples and the substrate as summarised in Figure 3.2. Thus, after the addition of the serum or faecal samples, rat monoclonal antibody with specificity for either IgM, IgG_1, IgG_2 or IgA was added. The monoclonal antibody responses were tested by using anti-rat Ig (H&L) The details of individual assay blocking serum and indicator system are shown in Table 3.9.

Table 3.9 Reagent details for monoclonal antibody detection system assays in sheep and in cattle

Sheep	Assay	Blocking	indicator
	IgG1	normal Rabbit serum	Anti-rat Ig (H & L)
	IgG2	normal Rabbit serum	Anti-rat Ig (H & L)
	IgA	normal Rabbit serum	Anti-rat Ig (H & L)

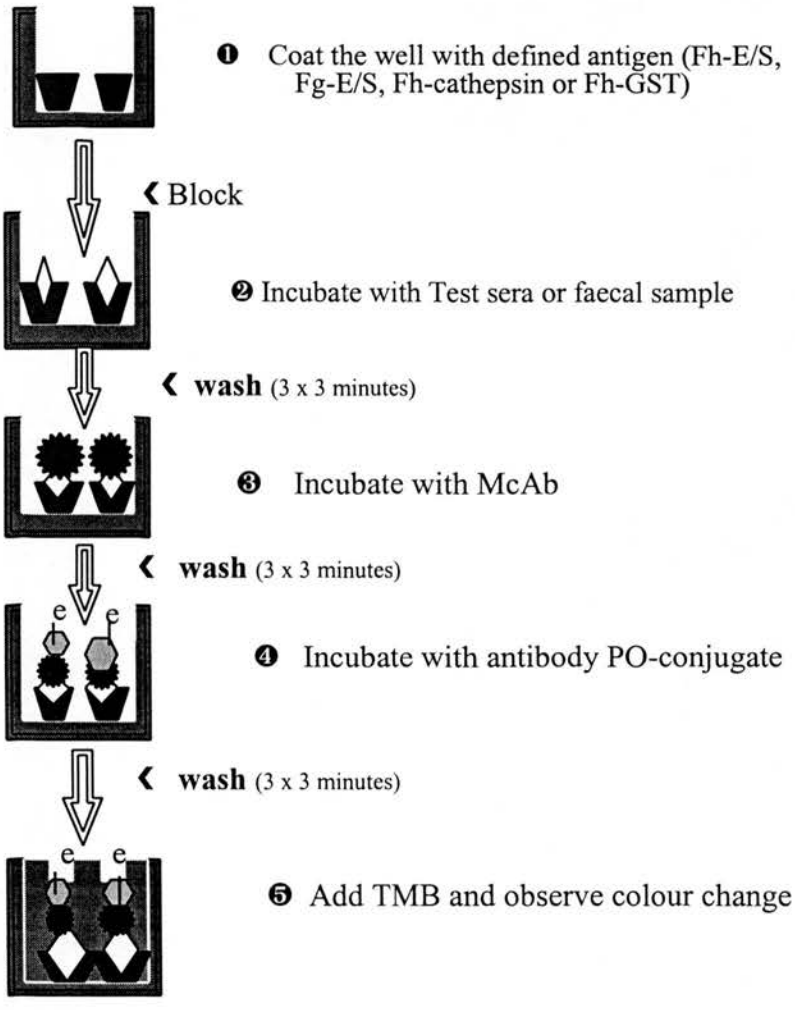


Figure 3.2: The indirect ELISA with monoclonal antibody detection system

Table 3.10: ELISA plate layout for the assay for sequential analysis.

	1	2	3	4	5	6	7	8	9	10	11	12
a	N	T-2	T-2	T0	T0	P	T1	T1				
b							N					P
c	B					B						
d							P					B
e	P					N						
f							B					N
g	N					P						
h							B					B

Key

P = Positive control sera
N = Negative control sera
B = Diluent (serum blank)
T-2, T0, etc. = Weeks post infection with either *F. hepatica* or *F. gigantica*.

3.7.4 Titration

Before carrying out sequential ELISA's chequerboard titrations of antigens test sera, monoclonal antibodies and conjugates were performed to determine the optimum concentrations. During monoclonal antibody titrations a comparison was made with Fh-E/S or Fg-E/S assays to monitor the assay and ascertain whether or not the assay was working for the respective immunoglobulin subclasses involved. Faecal samples were not titrated because they were in limited amount. The general indirect ELISA procedure described in sections 3.7.2 and 3.7.3 were used in the respective titrations.

3.7.5 ELISA Correction Factor

A correction factor was used to adjust the results of sequential screening for day to day and plate to plate variations. The correction factor always related back to the results of the first plate of assay particular sequence.

$$\text{Correction factor} = \frac{Po - No}{Pt - Nt}$$

Po = Average results of the positive sera from plate 1, day 1
No = Average results of the negative sera from plate 1, day 1
Pt = Average results of the positive sera from test plate
Nt = Average results of the negative sera from test plate

3.8 WESTERN BLOTTING (WB)

3.8.1 Sodium Dodecyl Sulphate Polyacrylmide Gel Electrophoresis (SDS-PAGE).

All the Western blotting studies were conducted using a discontinuous polyacrylmide gels consisting of a resolving or separating gel (lower) and a stacking (upper) gel buffer system (see appendix 1) as described by Laemmli (1970).

The samples were mixed with either reducing buffer or non reducing buffers (Appendix 1) at the ratio of 2 : 1 and boiled for 5 minutes at about 100 C. These samples were then cooled before being loaded in SDS-PAGE mini-gel (prepared according to the manufacturers specifications). 0.75 mm thick, mini-gels with 2 wells, small well for pre stained broad spectrum marker and the large well for samples, were used. Biotinylated broad spectrum molecular weight markers (8 ml) (Biorad Laboratories, code, 161-0319) were added to the marker well of the 2 wells and reduced or non reduced samples (300µl) were added to the larger well. The

samples together with the broad spectrum prestained markers were electrophoresis for 45 minutes at 200 Volts using 200/0.2 power supply (Bio-Rad®).

3.8.2 Electrophoretic Transfer Blotting

After electrophoresis, gels were electroblotted onto nitro-cellulose (NC) paper, Schleichler and Schuel) in a semi-dry blotter (Biorad Laboratories Homel Hempstead, UK). For each gel rectangular pieces of NC paper and filter paper (Whatman International Ltd.. Maidstone, Kent UK) were cut to a size slightly larger than the mini-gels and immersed in Bjerrum and Schaffer-Nielsen transfer buffer for 30 minutes (appendix 5). Three of the 6 wet filter papers were carefully placed on the flat surface of the negative plate of the transblot cell and the NC paper placed on top of the filter paper. The mini-gel was placed on top of the NC paper and then covered with the second wet filter paper. Precautions were taken to prevent trapping air. Two mini-gels prepared at one time. The cell was closed and electroblotting carried out for 45 minutes at 10 volts and a current limit of 0.26 amps per mini-gel. After transfer, the gel outline and the position of molecular weight markers on the gel were marked on the NCP. A mark to show whether reduced or non-reduced sample had been transferred onto the NCP was also placed on each mini blot.

3.8.3 Immunostaining

The sample arrangement of the sequential Western Blot is shown in Table 3.11. Following the protein transfer the mini-blots were washed 3 times in 0.05% v/v PBS/Tween 20®, each wash lasting 15 minutes. This washing procedure was used for all subsequent washing steps.

The reaction of transblotted fluke extracts with test sera was carried out in a mini PROTEAN II multiscreen apparatus (Biorad laboratories), which allows up to 20 serum samples on mini blots to be tested without having to cut the NC paper into strips. Two mini-blots, one containing reduced and the other non reduced extract were tested simultaneously. The mini-blots were aligned on the mini-PROTEAN II apparatus (figure 3). The optimum dilutions of serum biotinylated rabbit anti-bovine IgG, and streptavidin alkaline phosphatase were determined through titrations.

Free binding sites were blocked by 4% v/v normal goat serum (4% v/v NGS/PBS/Tween20®) in case of cattle serum examination and 4% normal rabbit serum (4% NRS/PBS/Tween20®) in case of sheep serum at 4^0C overnight. After a wash with 600 µl Tween20 per channel, test sera optimally diluted approprate blocking buffer was added and incubated at 37^0C for 1 hour. After the channels were washed and 600 µl per channel of biotinylated anti-bovine Ig (Sigma Chemicals, code B7140) diluted optimally in approprate blocking buffer was incubated at 37 C for one hour. After washing both the reduced and non-reduced mini blots were then removed from the mini-PROTEAN II apparatus, placed in plastic dishes where an adequate volume of an optimal dilution of streptavidin alkaline phosphatase conjugate (Amersham laborarories, code RPN 1234) was added to completely cover each mini blot. The mini-blots were incubated for 30 minutes at room temperature with gentle shaking and then washed. Adequate volumes of 5-bromo-4-chloro-3-indolyl-phosphate/nitroblue tetrazolium (BCIP/NPT) (Kirkegaard laboratories, code 50-81-07) to cover each mini blot were added to the plastic dishes and the dishes shaken gently on a shaker until bands appeared.

The reaction was stopped by washing the mini blots with distilled water. Blots were photographed using a Polaroid MP4 camera (no filter, f11, 1/8 seconds).

Table 3.11 The arrangement of channels of the mini-PROTEAN II apparatus and the alignment of the mini-blots for reaction with test sera.

Mr	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
T0	T1	T2	T3	T4														N	D

Mr = The area of the mini-blot containing molecular weight markers.
1, 2, etc. (Top) = The channels of the mini-PROTEAN II gasket
T1 (Bottom) = week after infection from which the serum sample in that channel was collected.
N (Bottom) = negative serum sample
D (Bottom) = serum diluent

3.8 SILVER STAINING

Samples for silver staining were prepared and run on SDS-PAGE gels (section 3.8.1) with some modifications. 1 mm thick, 10 well, ready made single percentage (10%)-slab gels (Biorad Laboratories code, 161-0907) were used. Biorad prestained broad range molecular weight markers (code, 161-0318) were added to wells 1 at 5 µl per well, while 45 µl per well of parasite extract samples were added to wells 2 to 10.

Following electrophoresis each gel was immersed for 30 minutes in a fixative containing 50% v/v methanol and 12% v/v glacial,acetic acid and 38% v/v of deionised distilled water. This solution was then replaced by one containing 10% v/v methanol, 5% v/v acetic acid and 85% v/v deionised distilled water and left to shake for 30 minutes. The gels were washed in distilled water for 20 minutes before being placed in Silver Nitrate solution of 0.95% w/v containing 5 ml of 0.8 nM NOAH, 3.5

ml of 35% v/v ammonium hydroxide and left on an orbital shaker for 20 minutes. After this stage the gels were again washed in distilled water v/v/v this time for 15 minutes and placed in 500 ml solution of 0.006 acetic acid, 125 µl of 38% formaldehyde and 50 ml ethanol. The gels were left on the shaker until the colour development was optimum. The reaction was stopped by placing the gels in 5%v/v acetic acid solution for one hour after which they were take for photographed, using a Polaroid MP4 camera.

3.9.4 Molecular Weight Estimation.

The distance migrated on the mini-blot by each of the molecular weight standards and the dye front of the detected antigens were measured and the relative mobility (Rf) value of each calculated using the formula:

$$Rf = \frac{\text{Distance migrated by molecular weight standard or antigen}}{\text{Distance migrated by the dye}}$$

The molecular weight (Log_{10}) of the standard (vertical axis) and the relative mobility of each standard (horizontal axis) were plotted (Fig. 3.3). The molecular weight of the detected antigens were determined by reference to the graph in relation to standard.

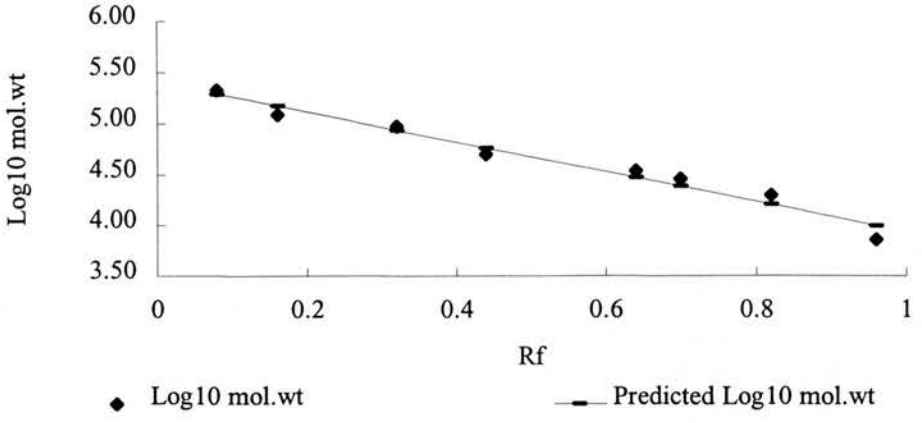


Figure 3.3 An example of molecular weight estimation by the Ferusons plot

3.10 DATA PROCESSING AND GRAPHICS

The collected data were processed and analysed by using a computer. The main software used are Microsoft® Word 6.0 and Microsoft® Excel version 5.0. Statistical analysis were done using Windows MINITAB® version 10Xtra (10.5).

The Student T-test and Manwhitney were used and $p < 0.05$ was considered significant.

CHAPTER FOUR

RESULTS

4.1 EXPERIMENTAL MONITORING

4.1.1 Experiment 1: *F. hepatica* (British and Peruvian strains) infection in sheep

Clinical Findings

All the sheep were clinically normal up to the 4 week post infection (wpi.), of the three sheep (5, 6 and 7) infected with 300 metacercariae (British strain) sheep 5 lost appetite was anaemic and often found recumbent by 6 wpi., sheep 5 deteriorated further by developing fever and dyspnoea by 8 wpi. and died at 11 wpi. Sheep 6 also lost appetite and became anaemic by 8 wpi. However, sheep 7 remained clinically healthy just as the uninfected control. The other two sheep, 9 and 10, infected with 200 Peruvian *F. hepatica* metacercariae showed no clinical abnormalities.

Live weight

The changes in live weight of individual sheep is shown in Figure 4.1a The live weight depression in sheep infected with 300 metacercariae (British strain) was between 6-11 wpi., thereafter it started to pick up. The infected sheep weight gain was much slower than uninfected control. Sheep 6 lost 3 kg by 9 wpi. Sheep 9 infected with 200 cysts was least affected. Data is in Appendix Table 4.1.

Parasitology

At the start of experiment sheep 5, 6, 7 and 8 were infected with nematode (Appendix Table 4.1) but the EPG values reduced sharply after deworming at -2 wpi.

The *F. hepatica* prepatent period ranged from 10 - 12 wpi. (Figure 4.1c) with egg counts peaking by sixteen wpi. Sheep 5 died before infection reached patency. Sheep (6 and 7) infected with 300 metacercariae had higher faecal egg count than those infected with 200 metacercariae.

Sheep 9 and 10 were not sacrificed at the end of experiment and left as egg donors for production of future infective materials. Thus only sheep 5 to 8 were sacrificed. The flukes recovery rate (Table 4.1) ranged from 73% to 33%. A small number of flukes in sheep 5 were still in the liver parenchyma the majority however were in the major bile ducts.

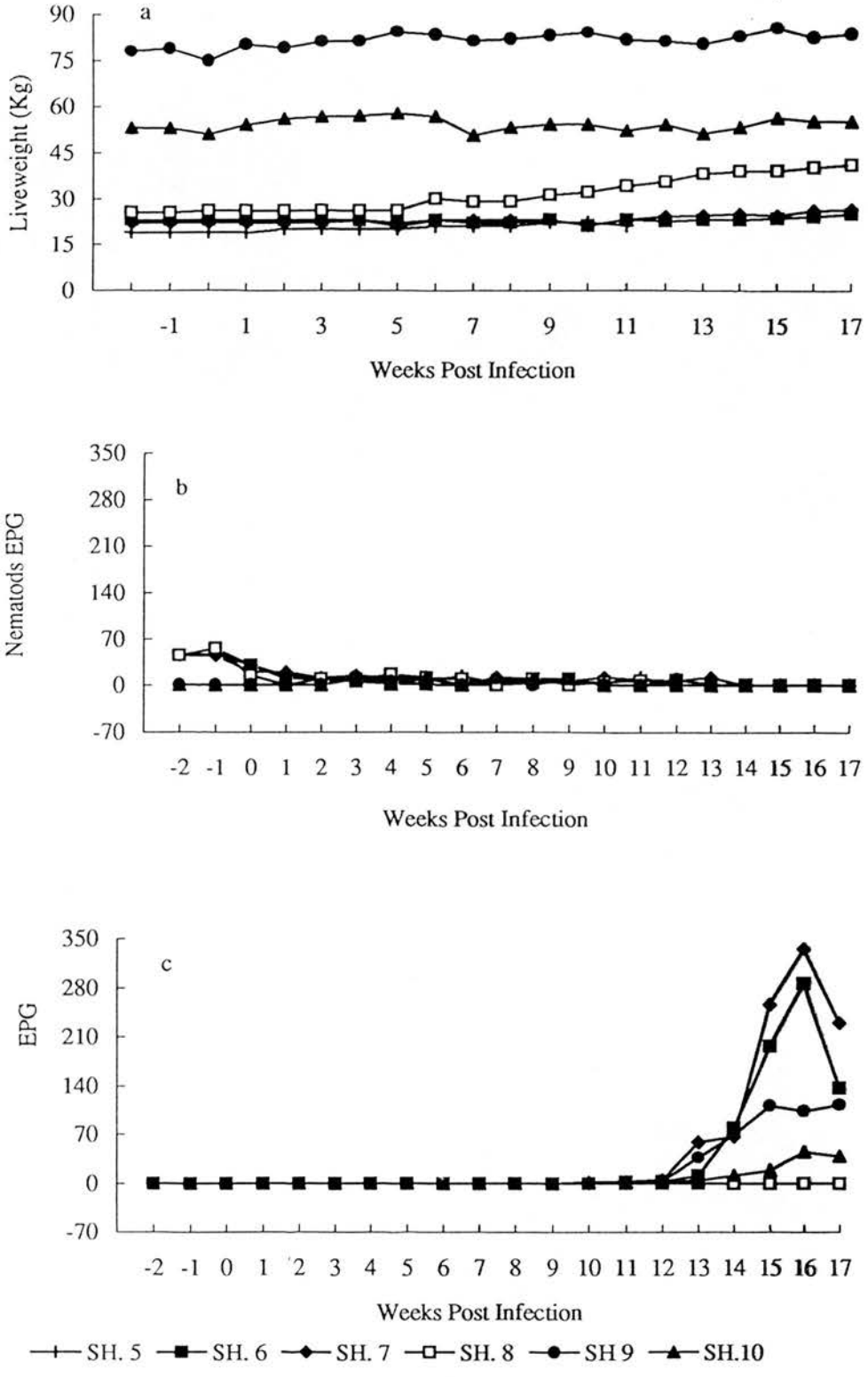


Figure 4.1: Liveweight (a) Nematodes EPG (b) and *F. hepatica* faecal EPG (c) Counts of sheep 5, 6 and 7 infected with 300 metacercariae, sheep 9 and 10 infected wtih 200 metacercariae. and uninfected control sheep 8.

Table 4.1: Experiment 1: Parasitological details of sheep infected with *F. hepatica* (British and Peruvian strains)

Sheep		Number of cysts given	Prepatent Period Wpi.	Flukes Recovered Number	%
5	British strain	300	†	75	33
6		300	11	150	50
7		300	10	172	73
8	Uninfected	0	-	-	
9	Peruvian strain	200	10	ND	
10		200	12	ND	

† = Died before patency
ND = Fluke Egg donors, not culled

Pathology

Lesions attributed to *F. hepatica* were wide-spread in sheep 5 and 6. The carcasses of these sheep exhibited pale membranes and loss of condition. The peritoneal cavity of the infected animals (Sheep 5, 6 and 7) had large amounts of serous fluid and the abdominal organs were adhered to the wall cavity (fibrinous peritonitis). The livers of infected sheep were enlarged with the major bile ducts extended. The left lobe was seriously affected leading to the development of abscesses (Figures.4.2). The hepatic lymph nodes were also enlarged. Pathohistology revealed a mild mononuclear cell infiltration (lymphocytes, plasma cells and macrophages). There was also necrosis and deposition of pigment substances as shown in Figure 4.3.

Figure 4.2: Liver of sheep 8 uninfected control (top left): Liver from sheep 7 infected with 300 metacercariae (top right) showing oedema of the entire liver, enlargement of gall bladder and distended bile ducts and liver (bottom) from sheep 6 also infected with 300 metacercariae showing hyperplasia and abscesses.

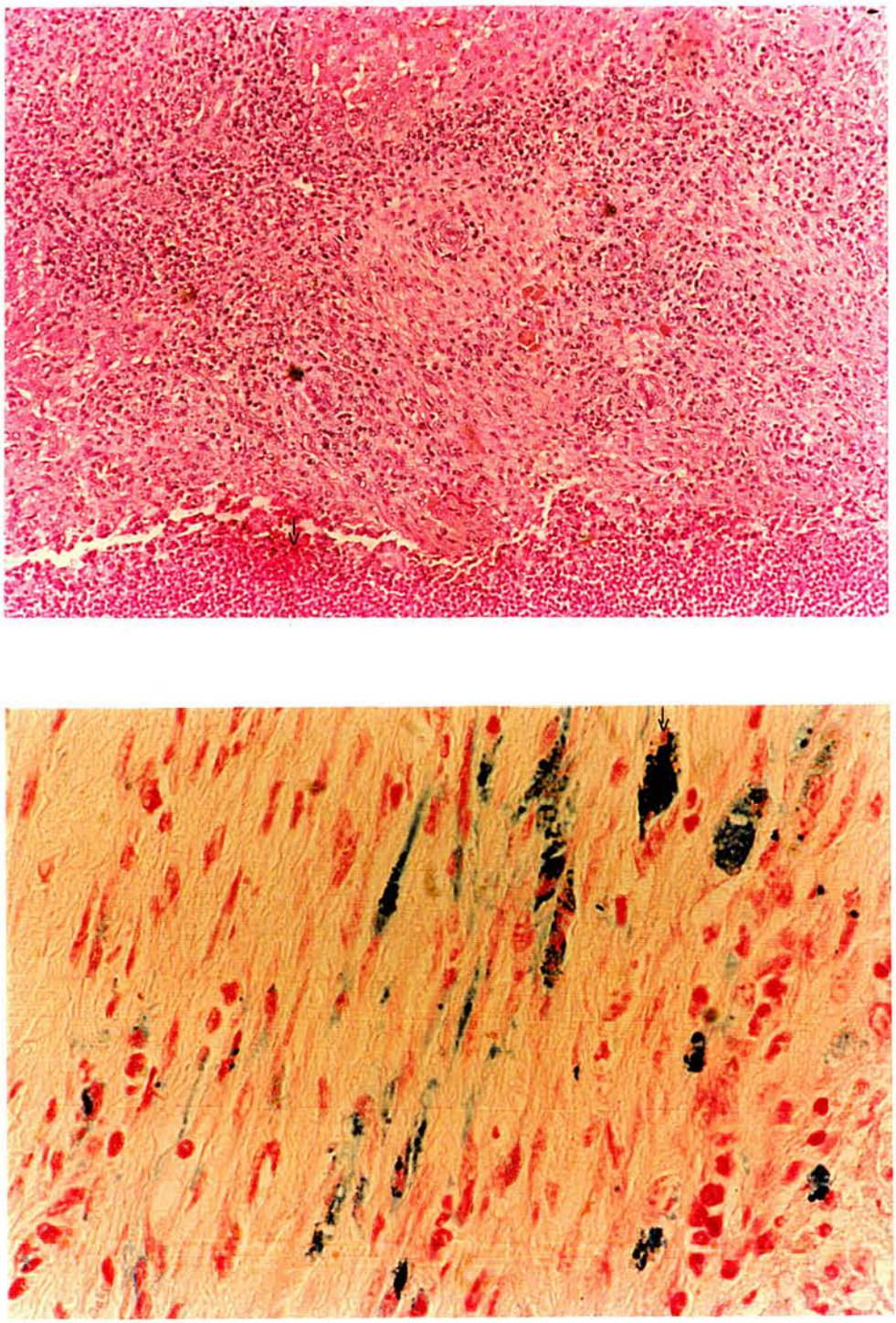


Figure 4.3: Photomicrographs of sections liver of sheep 5 infected with 300 metacercariae and died in 11 wpi. (top) showing severe necrosis of hepatocytes (H&E stained) and bottom a section from sheep 7 showing pigment deposits and fibrosis (H&E stained, x260 magnification).

Haematology

The sheep infected with 300 metacercariae exhibited anaemia, a reduction in values for red blood cell (RBC) counts, PCVs and haemoglobin concentration (Hb) compared to the uninfected controls while the two sheep infected with 200 metacercariae only showed reduced (RBCs). The values started a progressive decline by 5-7 wpi. (Figures 4.4a-c and Appendix Tables 4.2). mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) values indicated a clear increment in sheep 9 and 10 with a slight increment in sheep 6 and there was little changes in mean corpuscular haemoglobin concentration (MCHC) values (Figures 4.5a-c and Appendix Table 4.3) as a result of infection.

The total WBC and Eosinophil Counts increased in second wpi. and remained higher than the uninfected control (sheep 8) throughout the experiment. The differential white blood cell counts also a slight increase in neutrophils (Figures 4.6a-c) in infected sheep but the rest of the leukocytes (Lymphocytes, Monocytes and Basophils) were not elevated (see Appendix 4.4-4.6).

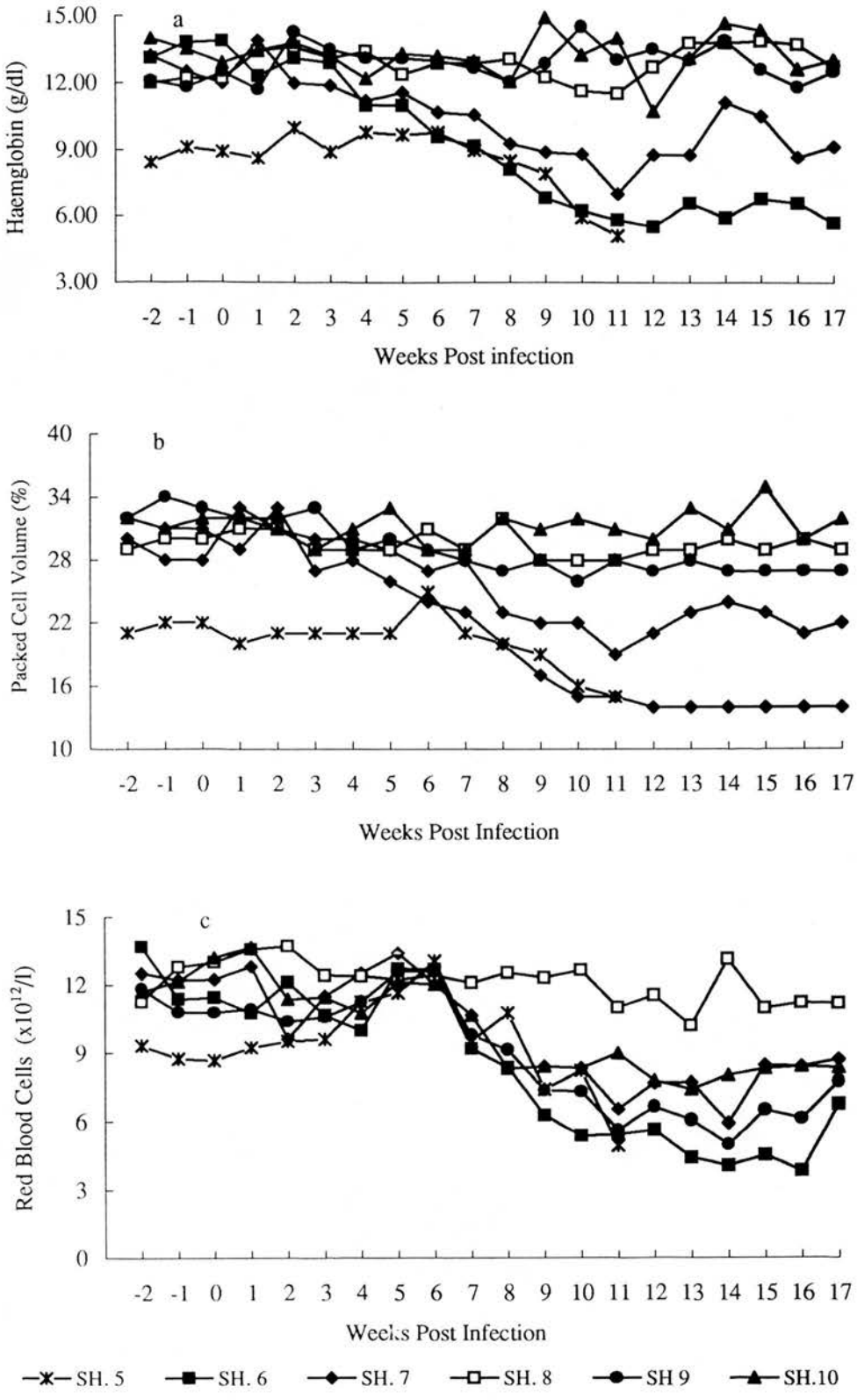


Figure 4.4: Haemoglobin (a) Packed Cell Volume (b) and Red blood cell (c) of 5, 6 and 7 sheep infected with 300 metacercariae, sheep 9 and 10 infected with 200 metacercariaeand uninfected control sheep 8

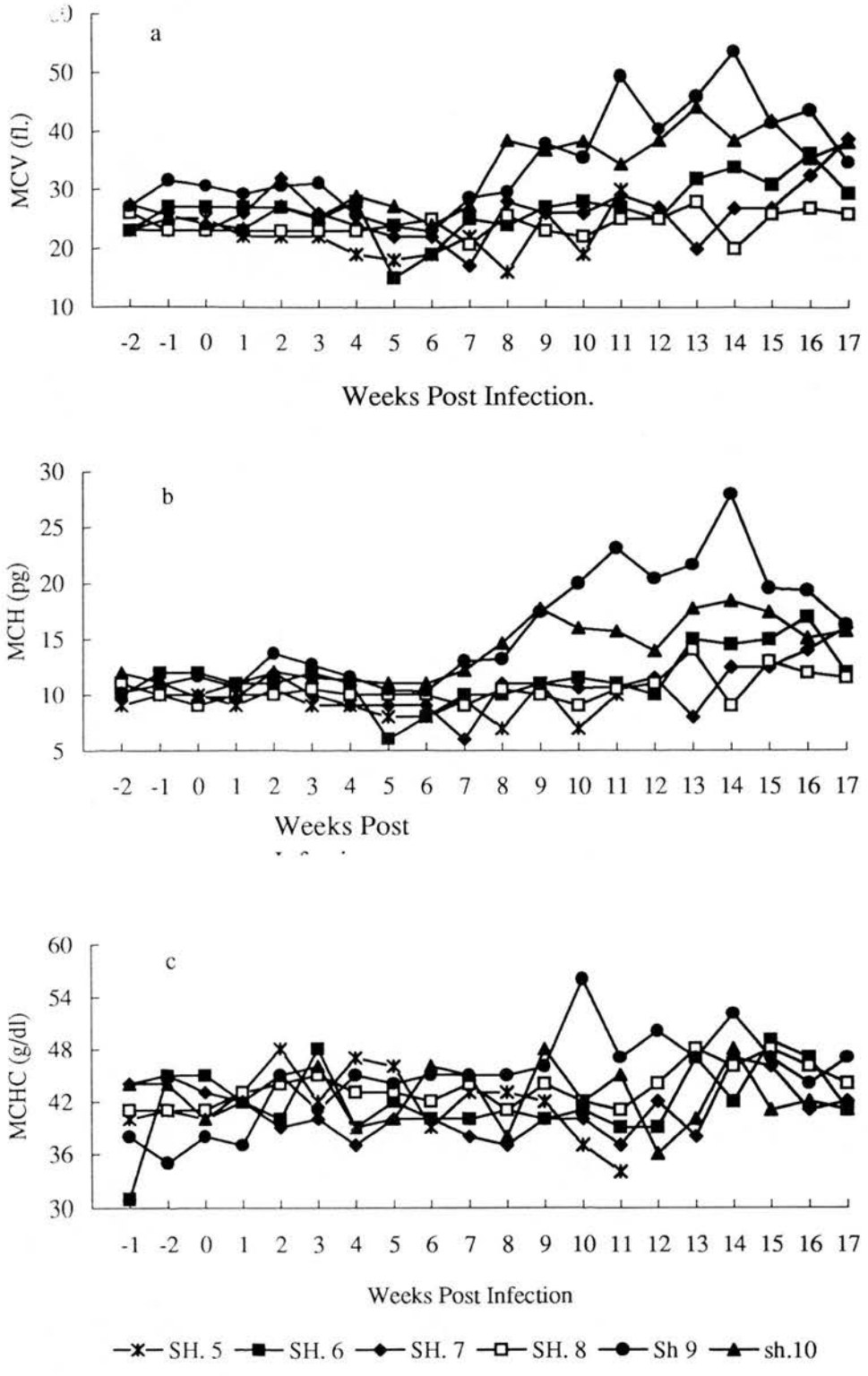


Figure 4.5: Mean Corpuscular Volume (MCV) (a), Mean Corpuscular Heamoglobin (MCH) (b) and Mean Corpuscular Heamoglobin Concentration (MCHC) (c) of sheep 5, 6 and 7 infected with 300 metacercariae sheep 9 and 10 infected with 200 metacercariae and as uninfected control sheep 8.

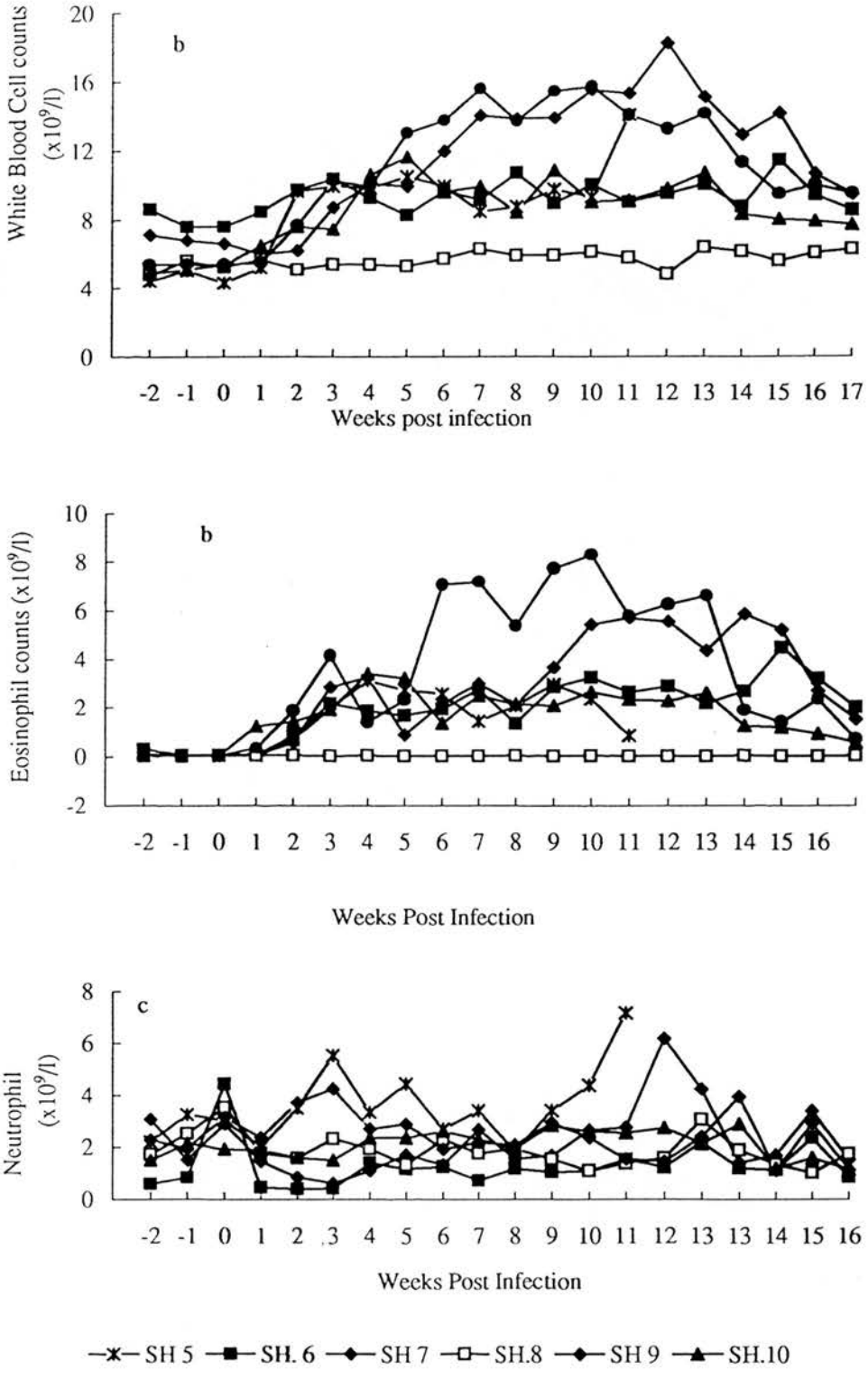


Figure 4.6: White blood cell counts (a), Eosinophil counts (b) and neutrophils counts (c) ($x10^9$/l) of sheep, 5, 6 and 7 infected with 300 metacercariae, sheep 9 and 10 infected wtih 200 metacercariae and uninfected control sheep 8.

Clinical Biochemistry

A reduction in albumin values was first noted 9-10 wpi. The serum albumin levels reduction was more severe in the more heavily infected sheep. There was no marked changes in the total protein levels until 8-9 wpi. when the values in sheep 5, 6, and 7 increased before decreasing by 10-11 wpi. The values in sheep 9 and 10 also decreased by 14 wpi., however the reduction was slower than in other three infected animals (Figure 4.7) and appendix table 4.7).

The values in uninfected control, sheep 6, remained consistently low throughout the experiment. The serum GLDH levels in infected sheep rose sharply by 3 wpi. with sheep 6 and 7 activities peaking by 4 wpi. Sheep 7 remained high up to 9 wpi. Interestingly sheep 5 which died 11 wpi. had GLDH levels similar to sheep 9 and 10 (Figure 4.8a and Appendix table 4.7).

There was a small rise in serum γ-GT activities in infected sheep from 3-6 wpi. in sheep 5 and 6 while sheep 7, 9 and 10 values rose by 9-10 wpi. These activities reached the peak by week 12-14 of infection (Figure 4.8a-b. and Appendix Table 4.7). The γ-GT levels in all infected animals declined by 17 wpi.

There was little difference in glucose and β-Hydroxybutyrate levels between sheep infected with 200 cysts and the uninfected control, however glucose levels fell in sheep infected with 300 metacercariae significantly ($P<0.05$, Mann-Whitney test) from 6 wpi. Serum β-Hydroxybutyrate levels were elevated significantly ($P<0.05$) in sheep infected with 300 metacercariae after Mann-Whitney test (Figure 4.9 and appendix table 4.8).

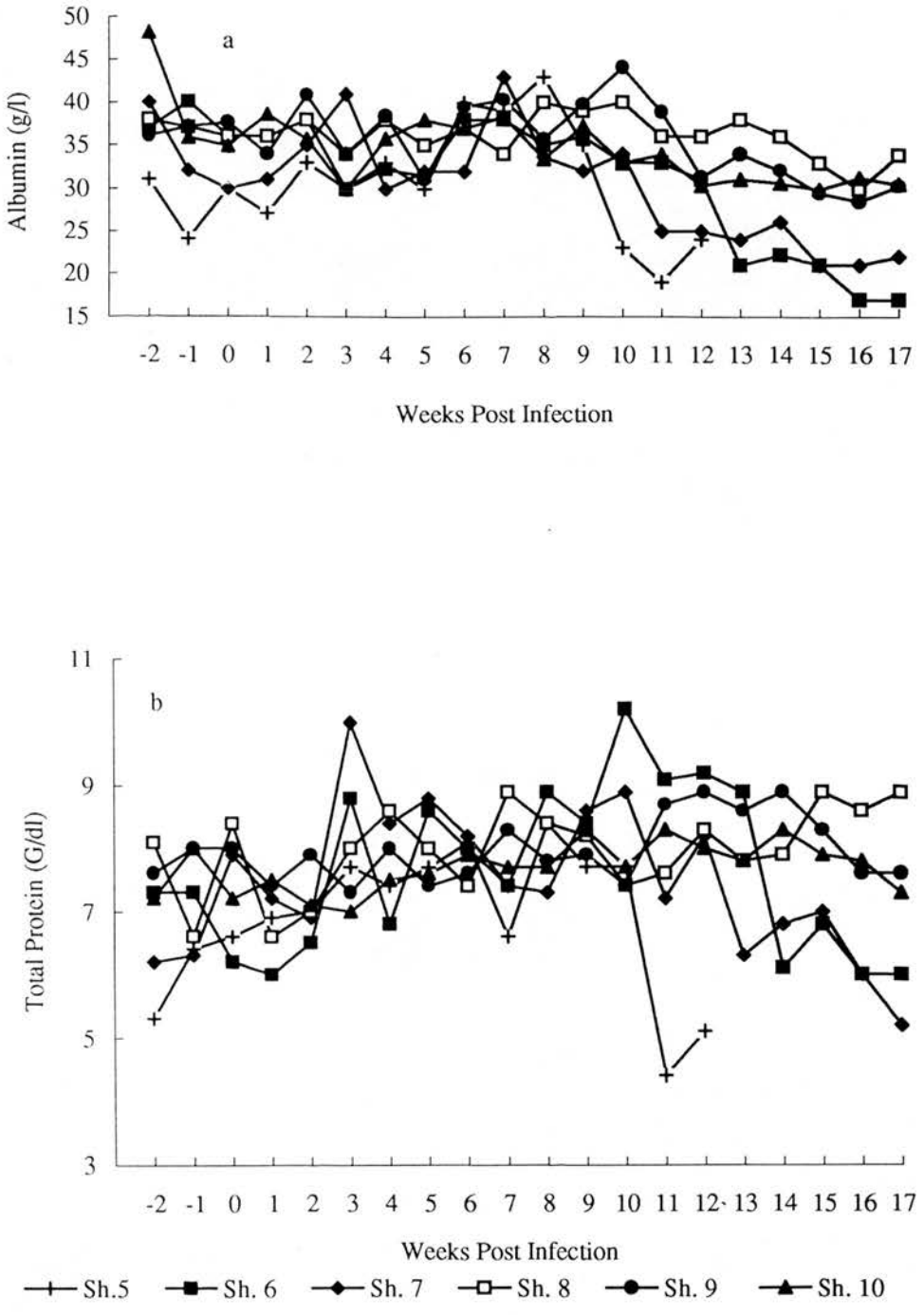


Figure 4.7: Albumin (a) and Total Protein estimates (b) of sheep 5, '6 and 7 infected with 300 metacercariae, sheep 9 and 10 infected with 200 metacercariae 'and uninfected control sheep 8.

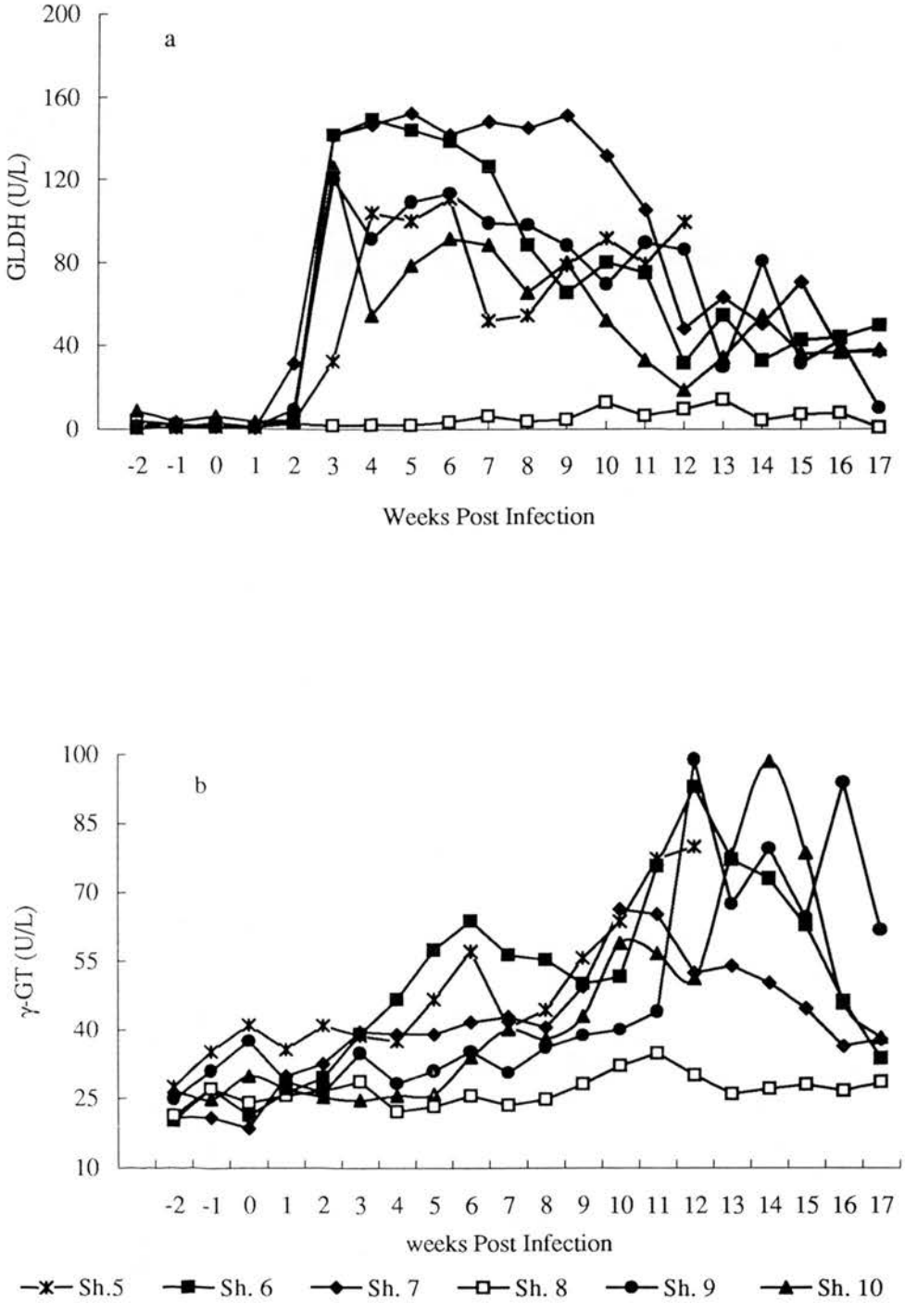


Figure 4.8: Glutamate Dehydrogenase (GLDH) (a) and Gamma Glutamyltransferase (γ-GT) (b) estimates of sheep 5, 6 and 7 infected with 300 metacercariae, sheep 9 and 10 with 200 metacercariae, and uninfected control sheep 8 .

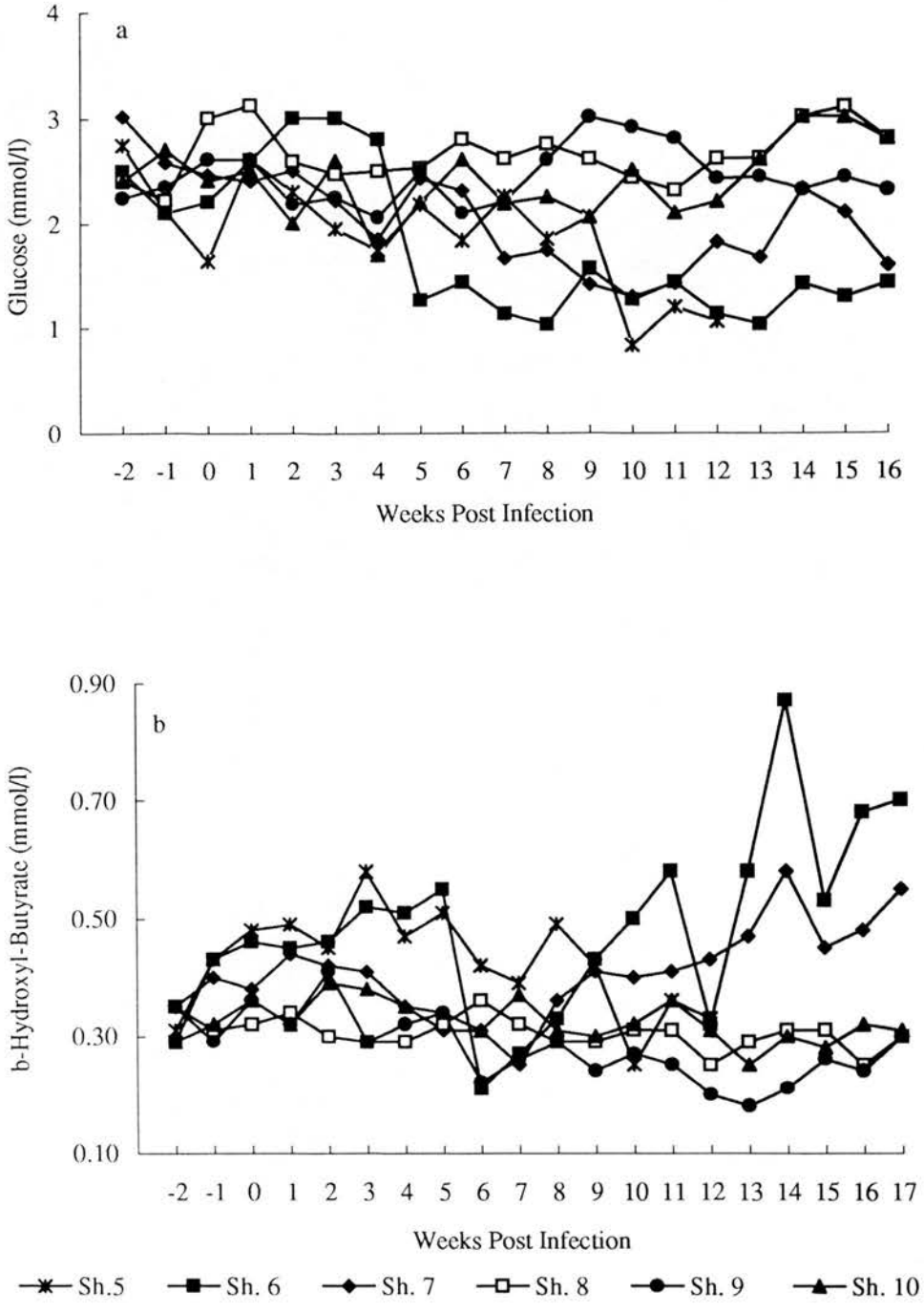


Figure 4.9: Glucose (a) and b-Hydroxyl-Butyrate (b) estimates of sheep 5, 6 and 7 infected with 300 metacercariae, 2 (9 and 10) sheep infected with 200 metacercariae and uninfected control sheep 8.

4.1.2 Experiment 2: *F. hepatica* (British strain) infection in sheep

This experiment was conducted by Mahato (1994). The monitoring included haematology clinical, biochemistry (excluding glucose and β-Hydroxybutyrate) and pathology.

Clinical Findings

All the sheep were clinically normal until 10 wpi. when sheep 26 (given 350 metacercariae) showed loss of appetite. None of the other sheep showed clinical signs (Mahato, 1993).

Live Weight

The infected sheep lost weight between 8 and 11 wpi. and grew at a slower rate thereafter. However the infected sheep gained weight over the experimental period, although less than the uninfected control sheep (Mahato, 1993).

Parasitology

The recovery of flukes from *F. hepatica* infected sheep ranged from 51.3-70.6% (Table 4.2). All of them were adults when recovered from the livers of infected sheep at 20 wpi., except for sheep 26 in which a few flukes were still migrating in the liver parenchyma (Mahato, 1993).

Table 4.2: **Experiment 2**: Some parasitological details of sheep infected with *F. hepatica* (British strains) (From Mahato, 1993).

Procedure	Sheep No.	Prepatent (Weeks)	Fluke Recovered Numbers	%
Infected with 150 metacercariae	24	10	77	51.3
	28	10	95	63.3
Infected with 350 metacercariae	26	12	247	70.6
	30	10	203	58.0
Uninfected control	22	-	-	-
	32	-	-	-

Pathological Findings

The remaining sheep showed typical lesions of chronic fasciolosis with enlarged livers and bile ducts distended with flukes (Mahato, 1993).

Haematology

The infected sheep exhibited a reduction in RBCs counts, PCVs and Haemoglobin values compared to the uninfected controls. There were no changes in MCHC values as a result of infection

The total WBC and Eosinophil Counts increased in second wpi. and remained higher than the uninfected control (sheep 22 and 32) throughout the experiment (Mahato, 1993).

Clinical Biochemistry

The serum albumin and total protein were carried out by Mahato, 1993.

The values of GLDH in uninfected control, sheep 22 and 32, remained consistently low throughout the experimcnt. The serum GLDH levels in infected sheep rose sharply by 2 wpi. peaking by 3-4 wpi. for sheep 26 and 30, 10 wpi. for sheep 24 and 9 wpi. for sheep 28 (Mahato, 1993).

Although there was a transient rise in serum Gamma Glutamyltransferase (γ-GT) levels in infected sheep, this was not pronounced until 8-10 wpi. (Data not shown).

Slight changes were observed in serum Glucose and β-Hydroxybutyrate levels in sheep infected with *F. hepatica*. Infected sheep had slight reduction in serum Glucose levels from about 5 wpi. The serum β-Hydroxybutyrate

measurements were erratic however there was a clear increase in β-Hydroxybutyrate levels between 9 and 12 wpi. in sheep 24, 26, and 28 as shown in (figure 4.10). Details are in appendix table 4.9.

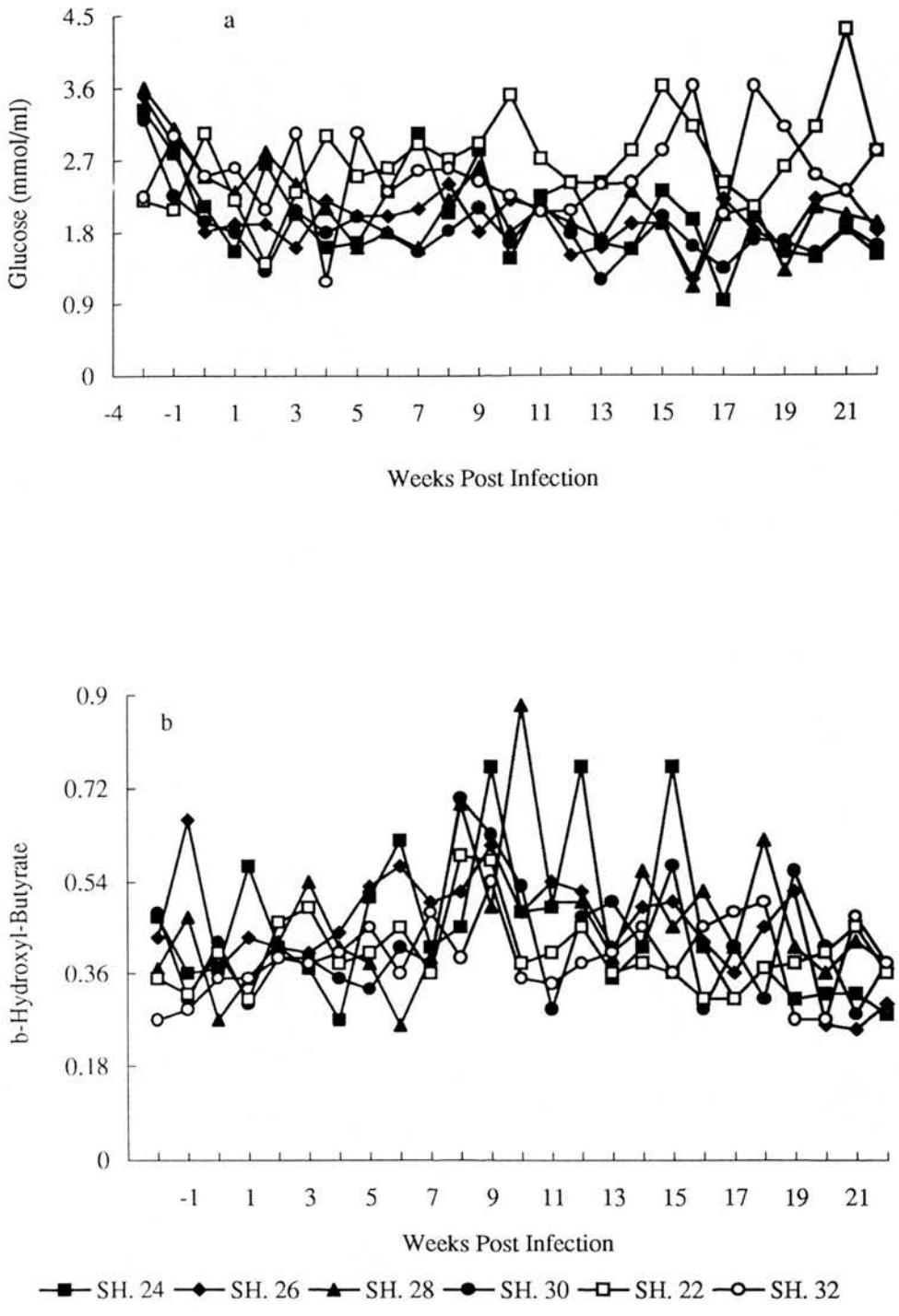


Figure 4.10: Glucose (a) and β-Hydroxyl-Butyrate (b) values in sheep 24, 26, 28 and 30 infected with *F. hepatica* and uninfected controls sheep 22 and 32 .

4.1.3 Experiment 3: *F. gigantica* (Kenyan strain) infection in sheep

Clinical Findings

All the nine sheep (Table 4.3) in this group were clinically normal until 13 wpi. when sheep 13 lost appetite, was anaemic and developed a temperature. The condition of this sheep did not improve and it was culled at 15 wpi. The condition of sheep 11 and 15 was considered poor from 6-7 wpi. but they recovered before the end of the experiment. Sheep 14 and 12 were least affected. The uninfected control sheep showed no clinical abnormalities.

Live Weight

A slight depression in mean live weight gain in infected sheep was first noted 5 wpi. but the condition improved by 12 wpi. This continued with minor fluctuations until the end of experiment (Figure.4.11a and Appendix Table 4.11).

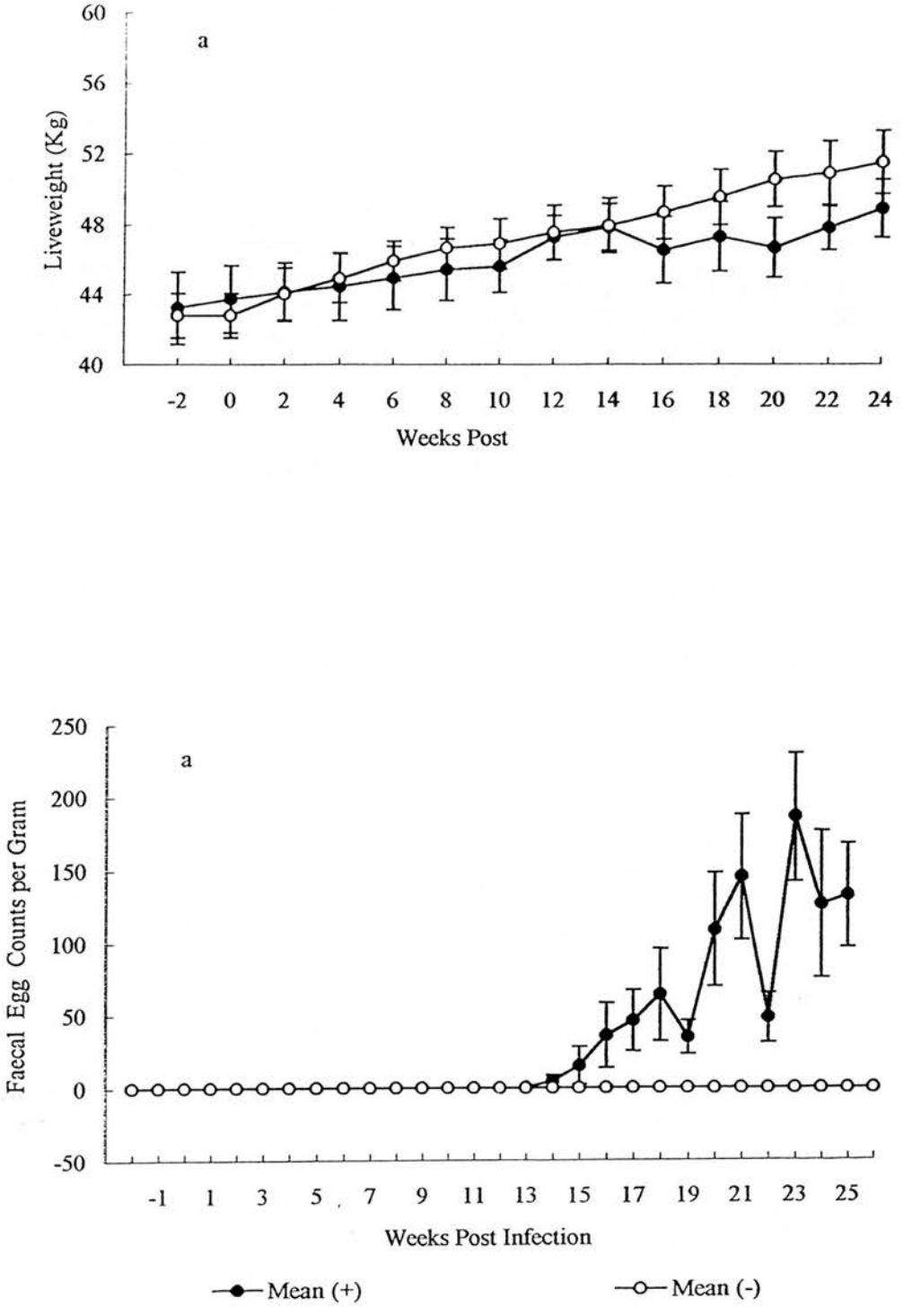


Figure 4.1 : Mean ±SEM Liveweight (a) and faecal egg counts (b) values of five sheep infected with 100 *F. gigantica* metacercariae (Kenyan strain) and four uninfected control sheep.

Parasitology

The sheep were dewormed at week -3 of the experiment and cleared of nematode infection. Patency was reached by 13-18 wpi. (Table 4.3) but sheep 13 however was culled before reaching patency and sheep 11 with the lightest fluke burden was the latest (18 wpi.) to shed parasite eggs and had the lowest peak EPG (Appendix Table 4.10).

Table 4.3: Some parasitological details of sheep infected with 100 metacercariae of *F. gigantica* (Kenyan strains)

Experiment 4	Sheep No.	Prepatent Period Weeks Post Infection Post	Flukes Recovered Number	%
Infected	11	18	34	34
	12	15	50	50
	13	culled before patency	45	45
	14	14	58	58
	15	13	76	76
Uninfected control	16	-	-	-
	17	-	-	-
	18	-	-	-
	19	-	-	-

Pathology

Lesions attributed to fasciolosis were most severe in sheep 12 and 13. The carcasses of these sheep exhibited pallor and emaciation. The peritoneal cavity of the infected Sheep 11 and 13 had large amounts of serous fluid. Sheep 15 organs were adhered to the abdominal wall displaying a classical fibrinous peritonitis (Figure 4.12**).** In sheep 12 the internal organs including the liver showed signs of adhesive peritonitis and liver fluke in the main bile duct (Figure 4.13). All the livers of infected sheep were enlarged with major bile ducts extended and hyperplastic lymph nodes. In sheep 11 the liver developed abscesses (Figure 4.14). The hepatic lymph nodes were also enlarged.

Pathohistology examination revealed a mononuclear cell infiltration as shown in Figure 4.16 (lymphocytes, plasma cells and macrophages). There was also infiltration of pigmentation in the liver parenchyma as shown in Figure 4.16. There was necrosis as shown in Figure 4.16 in a section through a minor bileduct of the liver from sheep 13 with a parasite eggs in it.

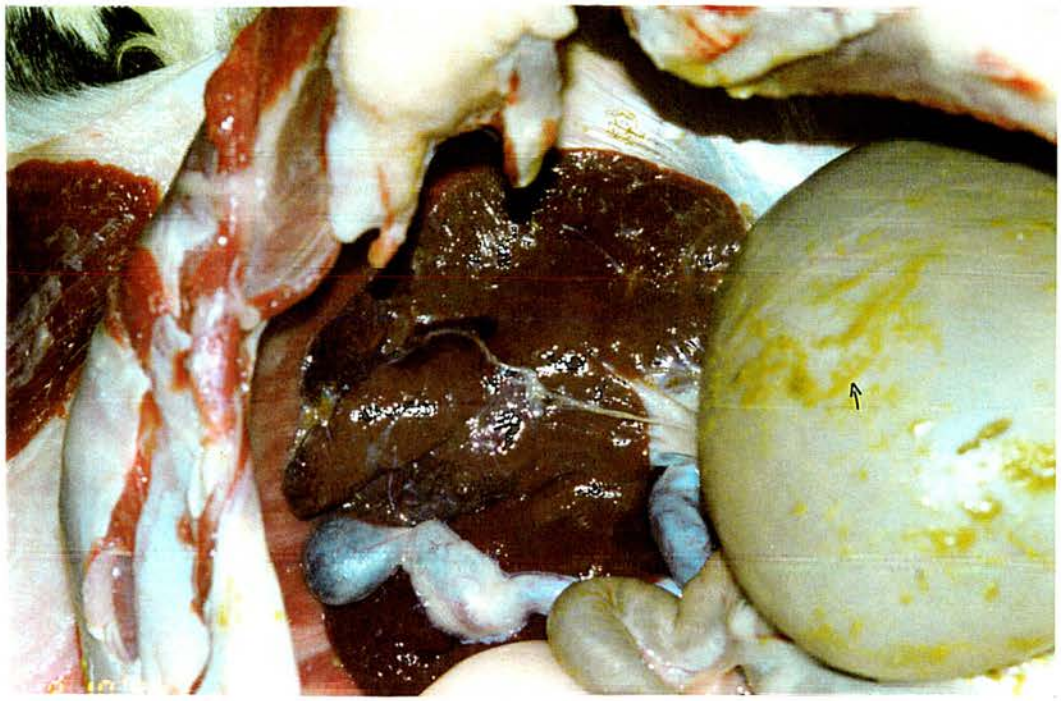


Figure 4.12: The internal organs with signs of adhesions (arrows) (top)

Figure 4.13: The infected liver (below) with flukes in the major bile duct (arrow) adhesive signs (arrow).

Figure 4.14: The infected liver with abscesses and necrosis on the surface (arrows).

Figure 4.15: Photomicrographs of the sections of liver from infected sheep 13: showing fasciola eggs trapped in the bile ducts (MSB stained x260 magnification)

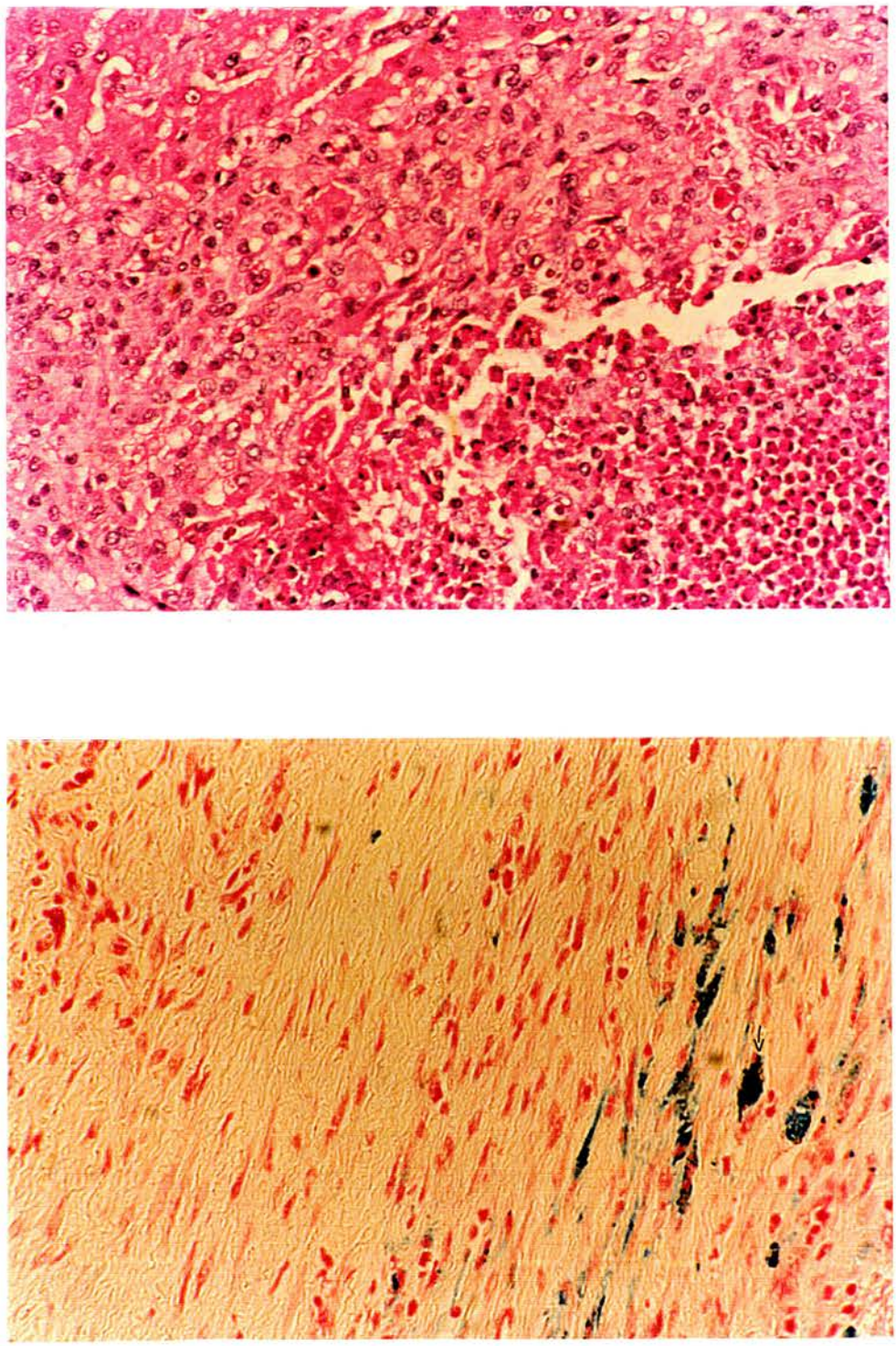


Figure 4.16: Photomicrographs of the sections of liver from infected sheep: showing large parenchyma necrosis with mass inflammatory cells infiltration (H&E stained x260 magnification) and bottom a section from sheep 15 showing pigment deposits (MSB stained x260 magnification).

Haematology

As judged by the RBC counts, Hb levels and PVC levels infected sheep developed an anaemia from 11 wpi. onwards. There was some variation between animals in the extent of the anaemia that developed but this did not relate to the parasite burden (Figure 4.17a-c and Appendix 4.12-14) for example sheep 11 with the lightest parasite burden had the greatest reduction in PVC.

The mean MCV and MCH values were elevated in the infected sheep but MCHC was apparently not affected (Figure 4.18 and Appendix table 4.4.15-17), this suggests that the animals developed normocytic normochromic anaemia.

The mean total white blood cell and eosinophil counts rose 2 wpi. and remaining higher throughout the experiment (Figure 4.19a-b). Mann-Whitney test confirmed that there already was significance ($p < 0.01$) in eosinophil response 2 wpi. between infected sheep and uninfected sheep. Although there was a slight increase in neutrophils (Figure 4.19c.) in infected sheep the rest of the leukocytes counts (lymphocytes, monocytes and basophils) were not elevated (Appendix table 4.18-4.24).

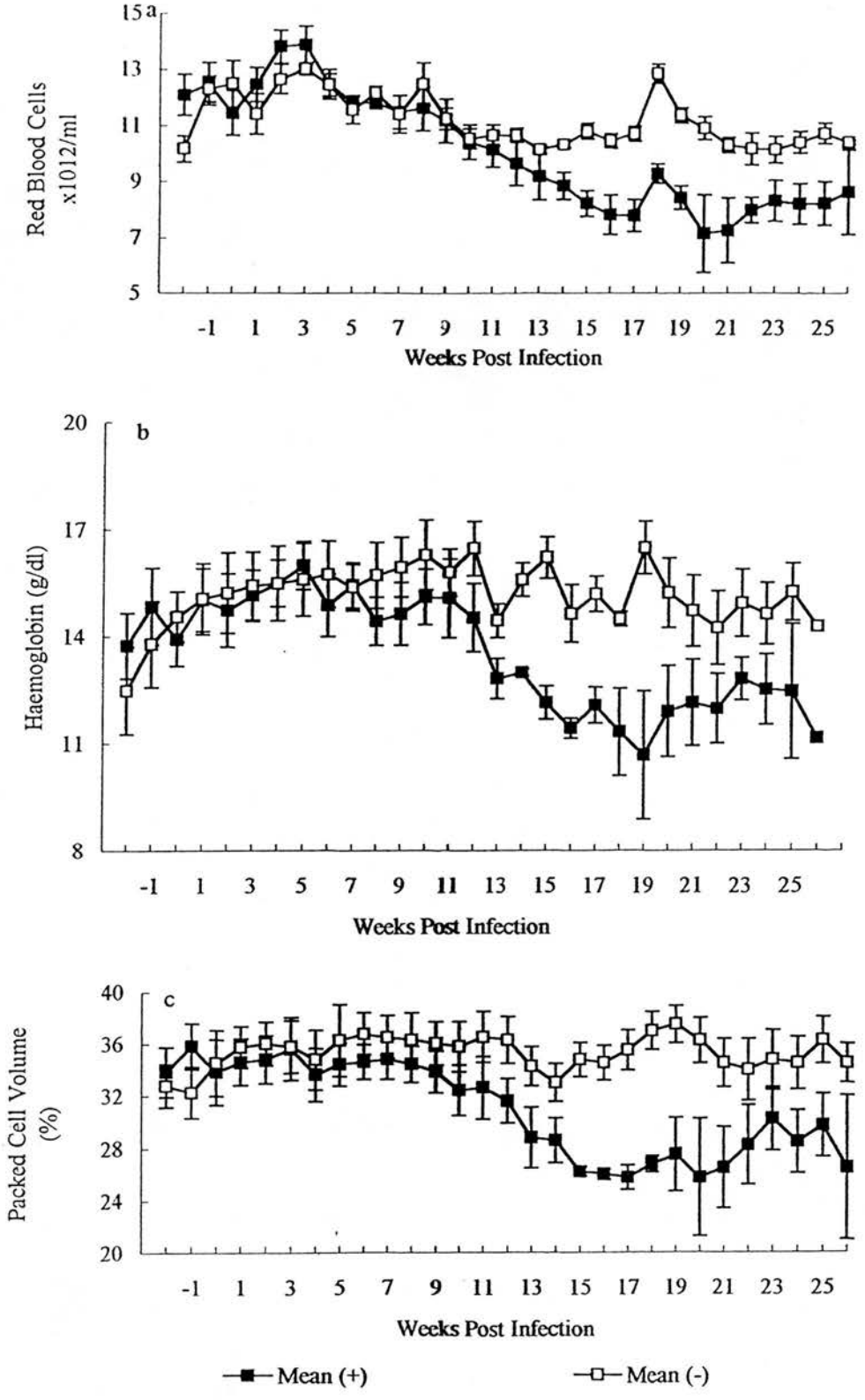


Figure 4.17: Mean ±SEM Red Blood Cells (a), Haemoglobin (b) and Packed Cell Volume (c) values of five sheep infected with 100 *F. gigantica* metacercariae (Kenyan strain) and four uninfected control sheep.

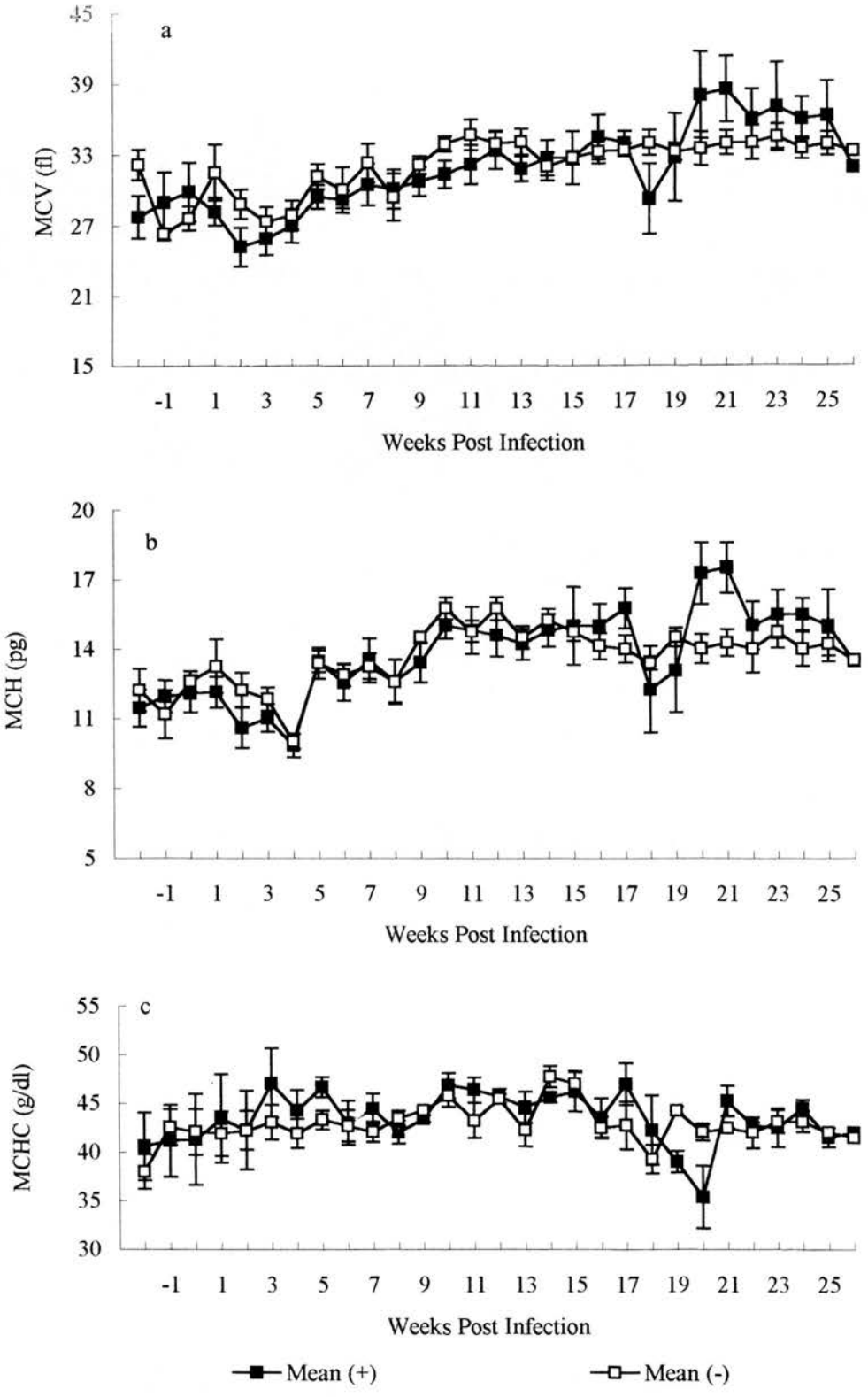


Figure 4.18: Mean ± SEM Mean Capscular Volume (MCV) (a), Mean Capscular Haemoglobin (MCH) (b) and Mean Capscular Haemoglobin concentration (MCHC) (c) of five Sheep infected with 100 *F. gigantica* metacercariae and four uninfected control sheep.

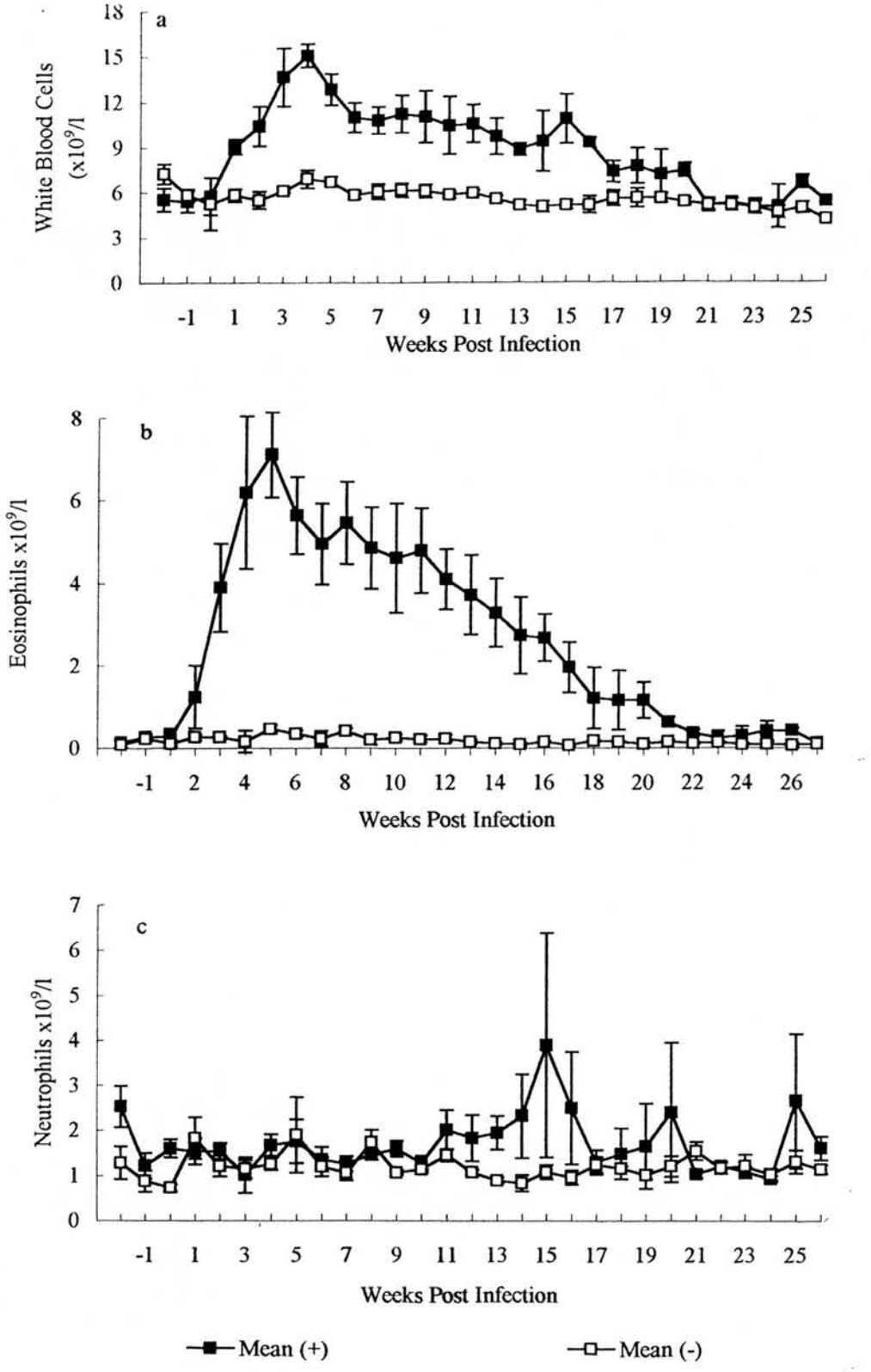


Figure 4.19: Mean ±SEM White Blood Cells (a), Eosinophils (b) and Neutrophils (c) values of five sheep infected with 100 *F. gigantica* metacercariae (Kenyan strain) and four uninfected control sheep.

Clinical Biochemistry

The serum total protein and albumin values are presented in Appendix Tables 4.25 & 4.26 and Figures 4.20a & b respectively. A reduction in mean serum albumin in infected animals was first observed by 4 wpi. but became marked by 11 wpi. There was a slight decrease in mean serum total protein levels in infected animals by 4 wpi. and became marked by 11 wpi. The mean serum protein levels in these infected sheep started to decline sharply by 17 wpi. reaching the minimum by 24 wpi.

A rise in mean serum GLDH activity levels in infected sheep was first noted at +2 wpi. peaking at 5 wpi. and remaining elevated until the end of the experiment. Mann-Whitney test confirmed this significant increse ($p < 0.05$) in serum GLDH activities by 2 wpi. in infected sheep compared to uninfected control sheep.

There was a slight transient rise in mean serum γ-GT activities in infected sheep from 5-8 wpi. The γ-GT levels peaked at 11-14 wpi. before declining slowly thereafter, and reaching the levels of uninfected control sheep by end of the experiment. In contrast to GLDH activities Mann-Whitney test showed that γ-GT activities were significant ($p < 0.05$) 2 wpi. in infected sheep compared to uninfected control sheep (Figure 4.21 and Appendix tables 4.27 & 4.28).

A reduction in glucose levels in infected sheep was apparent from 6-13 wpi. i.e. around the time of patancy but the levels became closer to normal sheep by 15 wpi. onwards. Mann-Whitney test confirmed that there was significant difference ($p < 0.05$) in glucose levels between 8 and 11 wpi. between infected sheep and uninfected sheep. The serum β-Hydroxybutyrate levels were elevated in infected

sheep from 7-17 weeks i.e. again around the time of patency (Figure 4.22 and appendix table 4.29 & 4.30). There was significant difference ($p < 0.05$) β-Hydroxybutyrate levels between 9 and 14 wpi. between infected sheep and uninfected sheep.

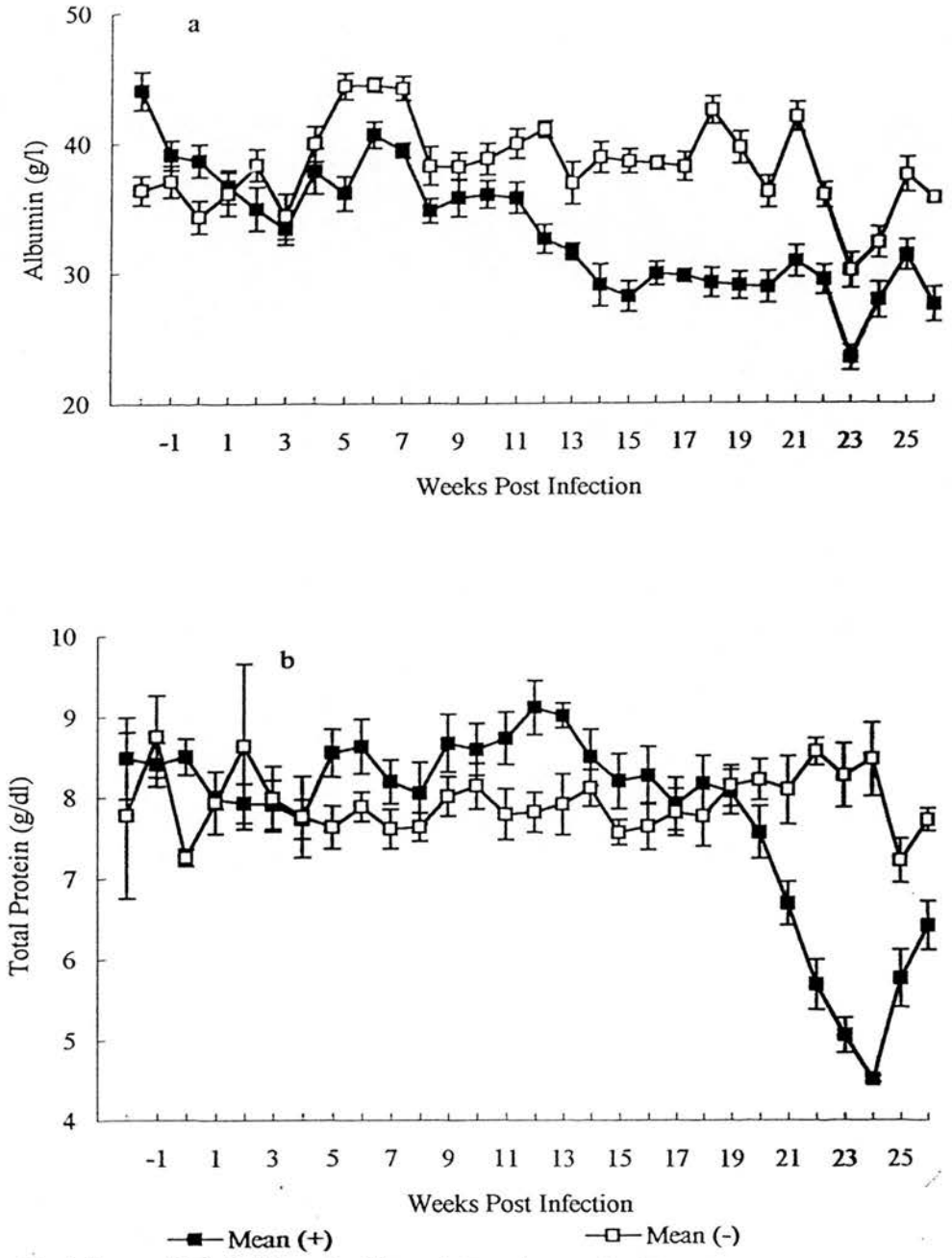


Figure 4.20: Mean ±SEM Albumin (a) and Total Protein (b) values of five sheep infected with 100 *F. gigantica* metacercariae (Kenyan strain) and four uninfected control sheep

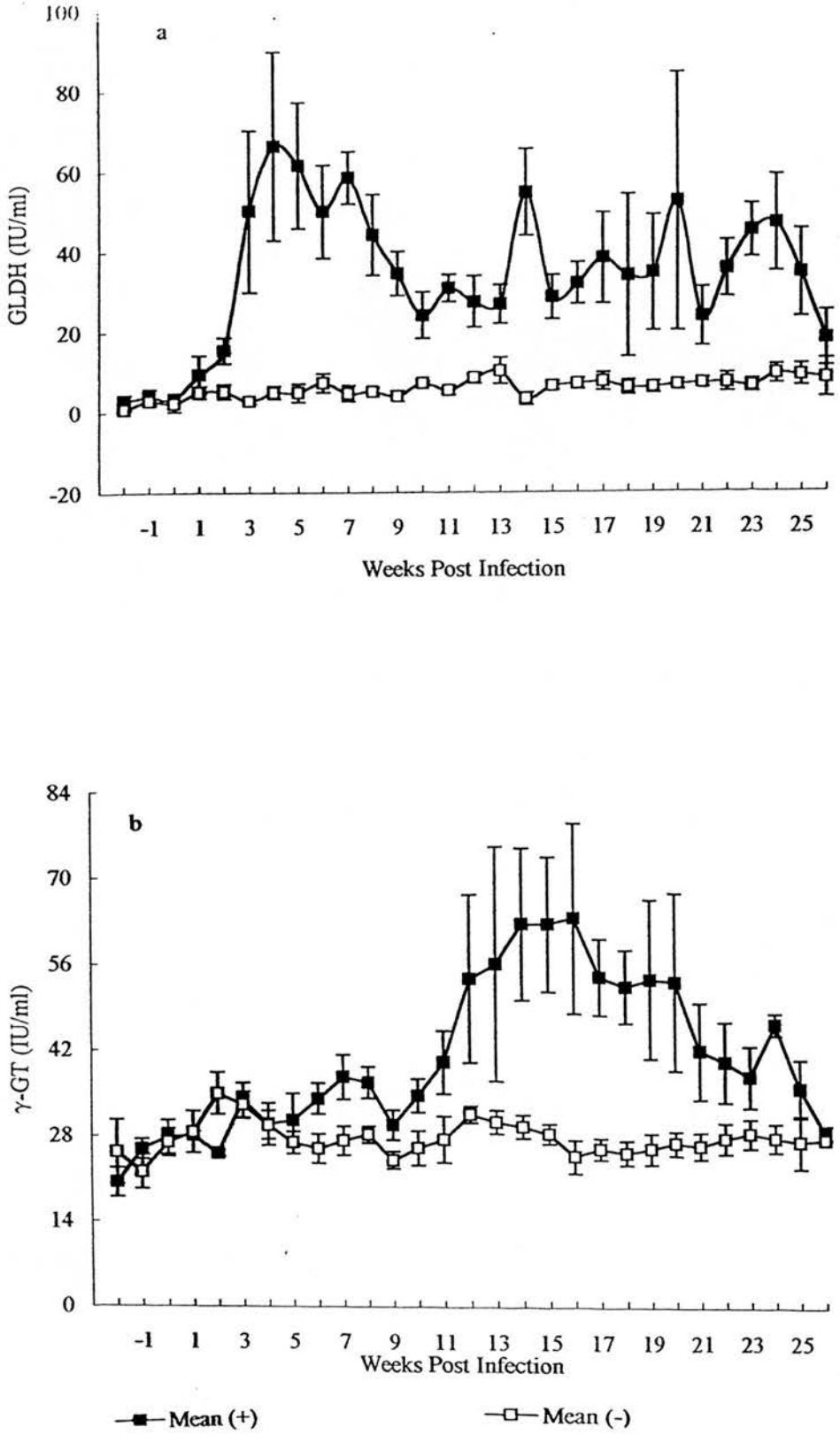


Figure 4.21: Mean ±SEM Glutamate Dehydrogenase (GLDH) (a) and Gamma Glutamyltranferase (γ-GT) (b) values of five sheep infected with 100 *F. gigantica* metacercariae (Kenyan strain) and four uninfected control sheep.

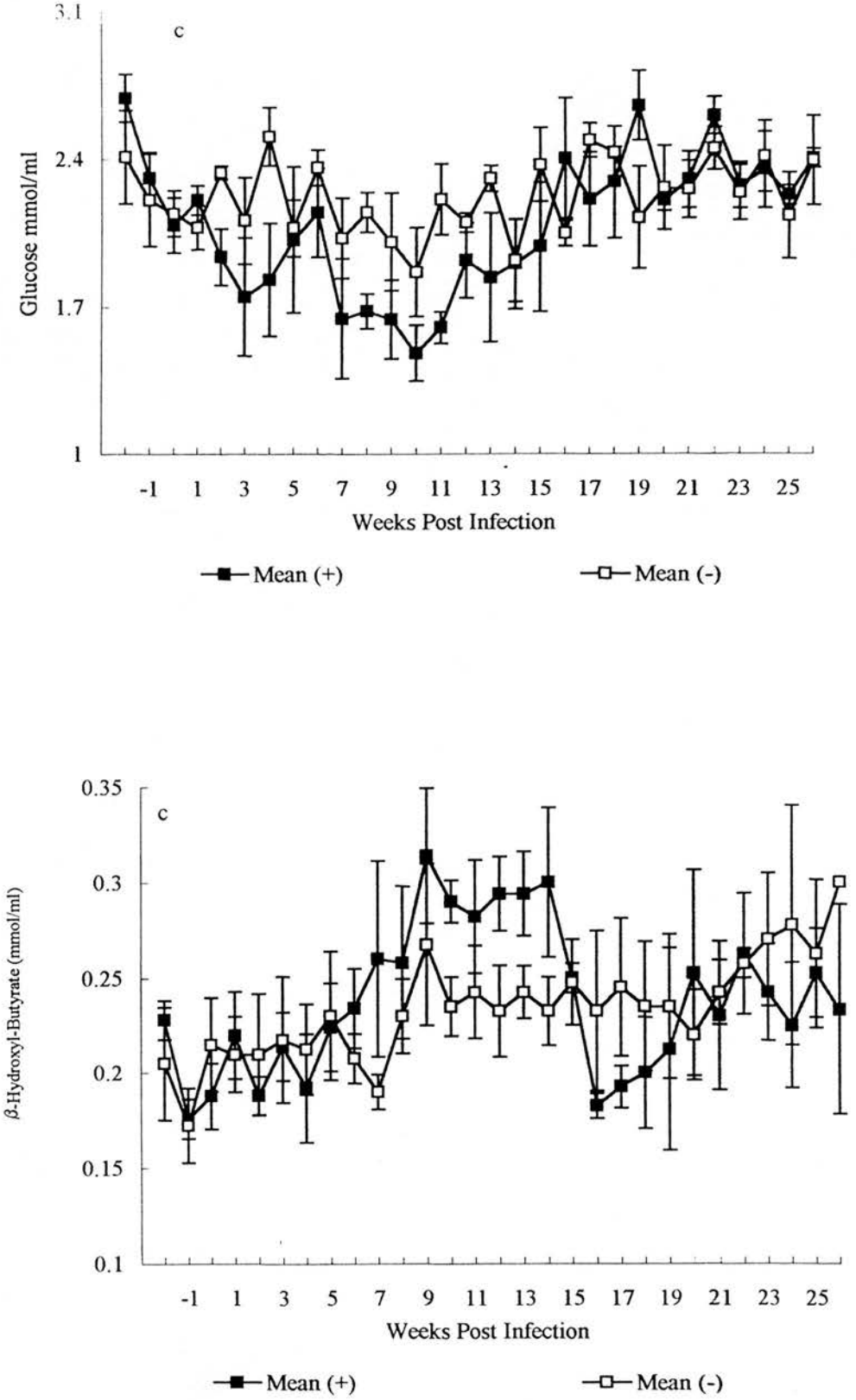


Figure 4.22: Mean ±SEM Glucose (a) and β-Hydroxybutyrate (b) values of five sheep infected with 100 *F. gigantica* metacercariae (Kenyan strain) and four uninfected control sheep.

4.1.4 Experiment 4: *F. Gigantica* (Kenyan strain) infection in sheep

This experimental group were monitored by Mahato (1993). The monitoring included haematology, clinical biochemistry (excluding glucose and β-Hydroxybutyrate) and pathology.

Clinical Findings

All the sheep were clinically normal until 11 wpi. Thereafter, sheep 23, 27 (given 150 metacercariae) and sheep 25 (given 350 metacercariae) had loss of appetite, abdominal distension and showed signs of anaemia. Sheep 23 died 14 wpi. and sheep 25 was culled 15 wpi. (Mahato, 1993).

Live weight

The infected sheep lost weight between 8 and 11 wpi. and grew at a slower rate thereafter. However the infected sheep gained weight over the experimental period, although less than the uninfected control sheep, except for sheep 27 (given 150 metacercariae) which lost 2.5 Kg (Mahato, 1993).

Parasitology

The recovery of flukes from *F. gigantica* infected sheep ranged from 9.7-55.3%, the lowest percentage being recovery from sheep 29 which had been given a higher infective dose of 350 metacercariae (Table 4.4). In sheep 23 and 25, more than 60% of the flukes were recovered from the liver parenchyma. Some ectopic flukes from sheep 25; 4 from the peritoneal cavity, 3 from the intestinal wall and 1 from the lungs were also recovered. In sheep 27 which was slaughtered at 20½ wpi.,

about 90% of the flukes were found in the bile ducts while 10% were still in the liver parenchyma. All the flukes recovered from sheep 29 were adults and located in the bile ducts. No flukes were recovered from the livers of any uninfected controls (Mahato, 1993).

Table 4.4: **Experiment 4:** Some parasitological details of sheep infected with *F. gigantica* (Kenyan strain) (Mahato, 1993).

Procedure	Sheep No.	Prepatent (Weeks)	Flukes Number	%
Infected with 1500 metacercariae	23	13	80	53.3
	25	13	83	55.3
Infected with 350 metacercariae	27	14	181	51.7
	29	13	34	9.7
Uninfected control	21	-	-	-
	31	-	-	-

Pathological Findings

Lesions were widespread in sheep 23 and 25. The carcasses of these sheep were pale; the peritoneal cavity contained large quantities of blood-stained fluid and blood clots; the small and large intestines were filled with blood. The livers were enlarged with prominent haemorragic tracts and with areas of fibrinous peritonitis on the surface. The gall bladders were enlarged and filled with clotted blood. The liver of sheep 23 was pale in colour than that of sheep 25 and there was a large blood clot between the liver capsule and the parenchyma. The lungs of sheep 25 showed large areas of congestion, these areas contained typical haemorragic tracts due to a migrating fluke which was found in the subpleura, also subpleural oedema was prominent.

The remaining sheep showed typical lesions of chronic fasciolosis with enlarged livers and bile ducts distended with flukes (Mahato, 1993).

Haematology

As judged by the RBC counts, Hb levels and PVC levels infected sheep developed an anaemia from 5-7 wpi. onwards. There was some variation between animals in the extend of the anaemia that developed but this did not relate to the parasite burden.

The mean MCV and MCH values were elevated in the infected sheep but MCHC was apparently not affected and this suggests that the animals developed normocytic normochromic anaemia.

The mean total white blood cell and eosinophil counts rose 2 wpi. and remaining higher throughout the experiment. Although there was a slight increase in infected sheep the rest of the leukocytes counts (lymphocytes, monocytes and basophils) were not elevated (Mahato, 1993).

Clinical Biochemistry

The serum albumin and total protein were done by Mahato, 1993. The values in uninfected control, sheep 21 and 31, remained consistently low throughout the experiment. The serum GLDH levels in infected sheep rose sharply by 2 wpi. peaking by 3-4 wpi. (Mahato, 1993).

Although there was a transient rise in serum Gamma Glutamyltransferase (γ-GT) levels in infected sheep, This was not pronounced until 11-12 wpi. (Mahato, 1993).

Slight changes were observed in serum glucose and β-Hydroxyl-butyrate levels in sheep infected with *F. gigantica*. Infected sheep had slight reduction in serum glucose levels from about 10 wpi. (Figure 4.23). There was an increase in β-

Hydroxyl-Butyrate levels however with a lot of variation within the infected sheep (Figure 4.23). Details are in appendix 4.31.

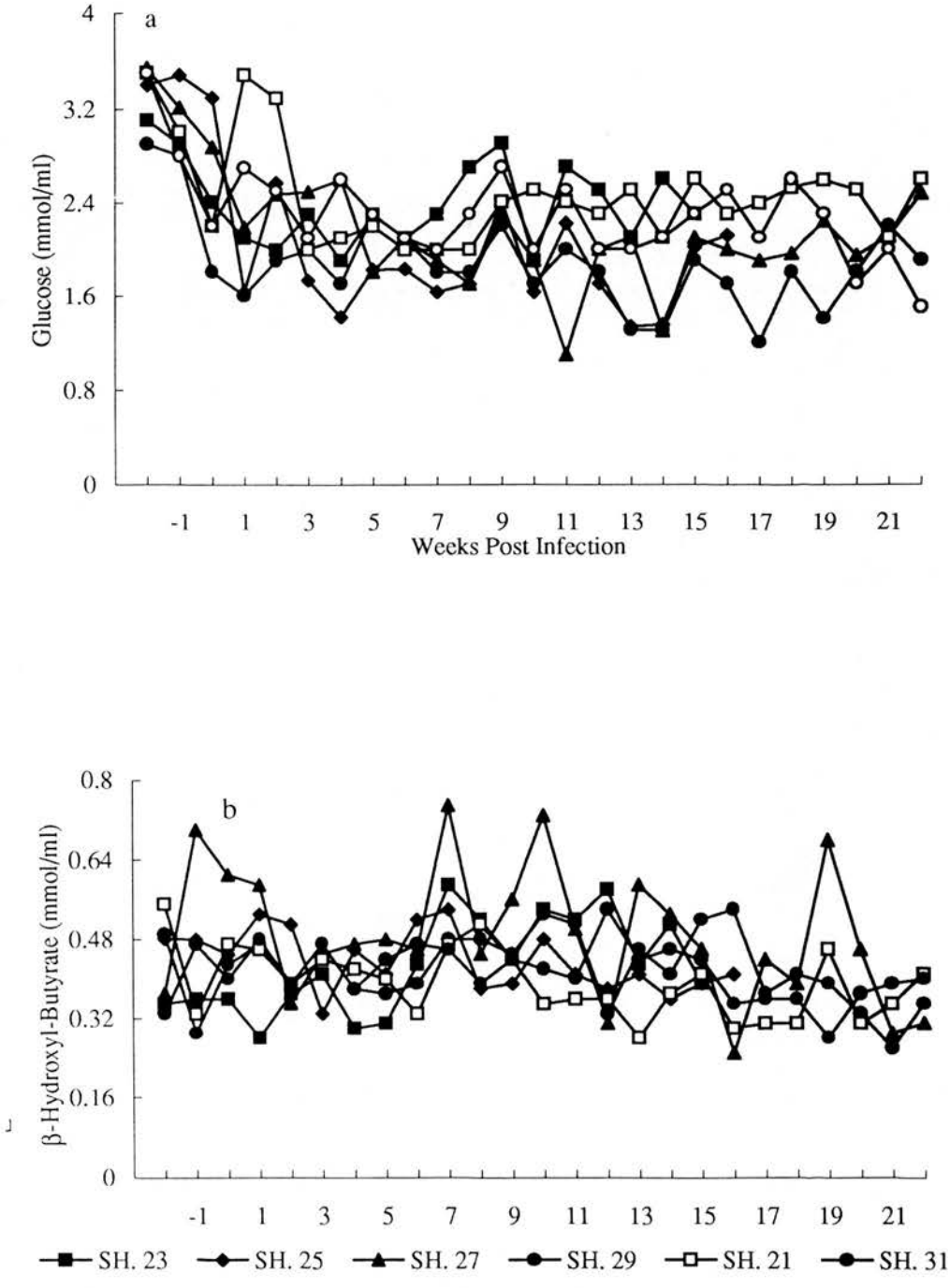


Figure 4.23: Glucose (a) and b-Hydroxyl-Butyrate (b) values in sheep 23, 25, 27 and 31 infected with *F. gigantica* and uninfected controls sheep 21 and 31.

4.1.5 Experiment 5: *F. hepatica* (Peruvian strains) infection in Cattle

Clinical Findings

Infected cattle displayed few clinical symptoms of fasciolosis. Calf 15 did show a mild loss of condition after challenge infection and calf 34c developed diarrhoea at 8 wpi. This diarrhoea seen in calf 34c lasted for two days and was not attributed to fasciolosis (Table 4.5).

Parasitology

Infected calves reached patency 8-11 wpi following primary infection (Figure.4.24). The EPG of infected and challenged calf 15c dropped to 0 by 8 weeks post challenge infection while eggs could still be detected in the faeces of the other infected calves. The fluke recoveries were very low (Table.4.5) with calves 14c with only primary infection and culled 38 wpi having the lowest flukes. The fluke recoveries in the challenge control calves 34c and 45c, were rather low at <7%.

Table 4.5: Experiment 5: Some parasitological details of calves infected with metacercariae of *F. hepatica* (Peruvian strain)

Procedure	Calf	Number of metacercariae administered at week No.			Prepatent Period	Flukes recoveries	
	No.	-25	-15	0	(Weeks)	No	%
Infection	14c	200	-	-	9	3 (2 dead)	1.5
Infection & challenge	15c	200	100	-	11	7	2.3
Infection & challenge	23c	-	600	100	9	15	2.1
Challenge control	34c	-	-	450	8	20	4.4
Challenge control	45c	-	-	450	8	30	6.7
Uninfected control	26c	-	-	-	-	0	

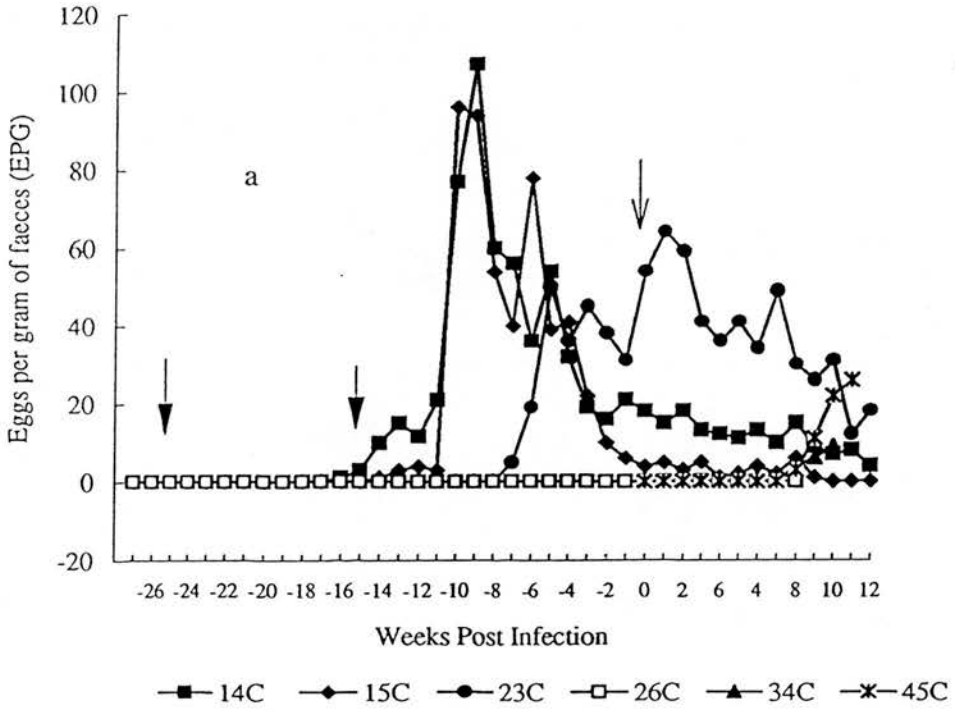


Figure 4.24: Eggs per gram (EPG) values in calves 14c, 34c and 45c with single *F. hepatica* (Peruvian strain) infection, calves 15c and 23c with challenge infection and calf 26c uninfected control.

← Primary infection ← Challenge infection

Pathological Findings

In all infected calves lesions were restricted to the livers. There was very little difference in appearance between calf 14c liver with a single infection and the uninfected calf 26c liver. This was consistent with light infection of calf 14c and the fact that *post-mortem* was done 38 wpi when the liver had almost fully regenerated. The liver of infected and challenged calf 23c on the other hand showed most severe congestion and calcification (Figure 4.25.). This is consistent with this calf having received the highest infection dose and challenge infection only 13 wpi before *post-mortem*.

After histology examination the sections from the challenge and infected calves 15c and 23c showed severe calcium deposits extending into the parenchyma. The sections from calf 15c also revealed strong fibrosis (Figure 4.26). Bile duct thickening and fluke eggs were most apparent in calf 23c (Figure 4.27). There was mononuclear cell infiltration (lymphocytes, plasma cells and macrophages) in the livers of infection and challenge calves 15c and 23c and challenge control calves 34c and 45c. This again is consistent with these calves having received light infection.

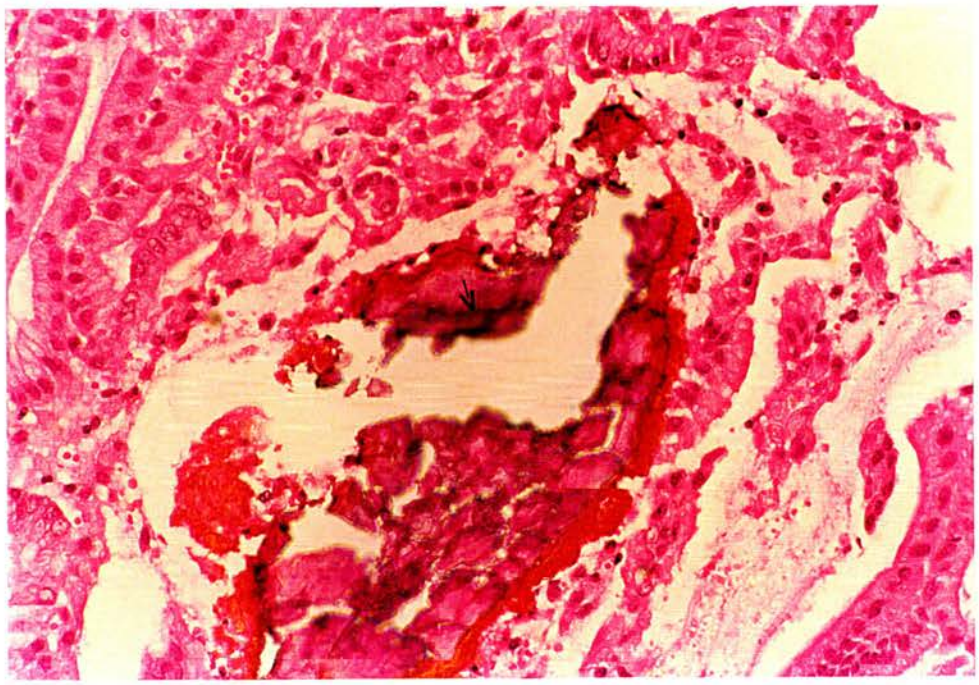


Figure 4.25: Photomicrograph of the section of liver from infected calf 23c: showing large calcification deposits in the bile duct (Arrow) (H&E stained x52 magnification)

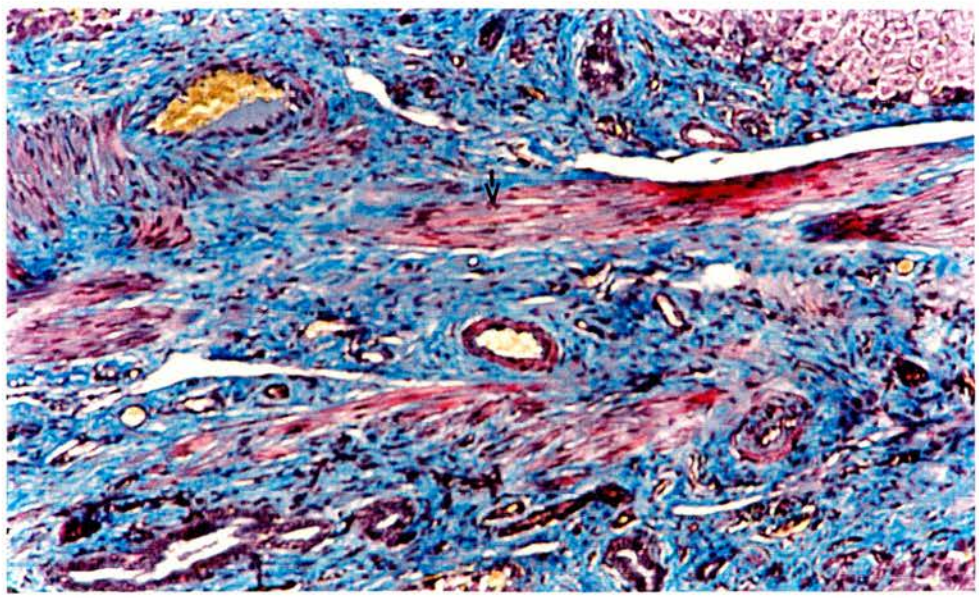


Figure 4.26: Photomicrograph of the section of liver from infected calf 15c: showing extensive fibrosis (Arrow) (H&E stained x52 magnification)

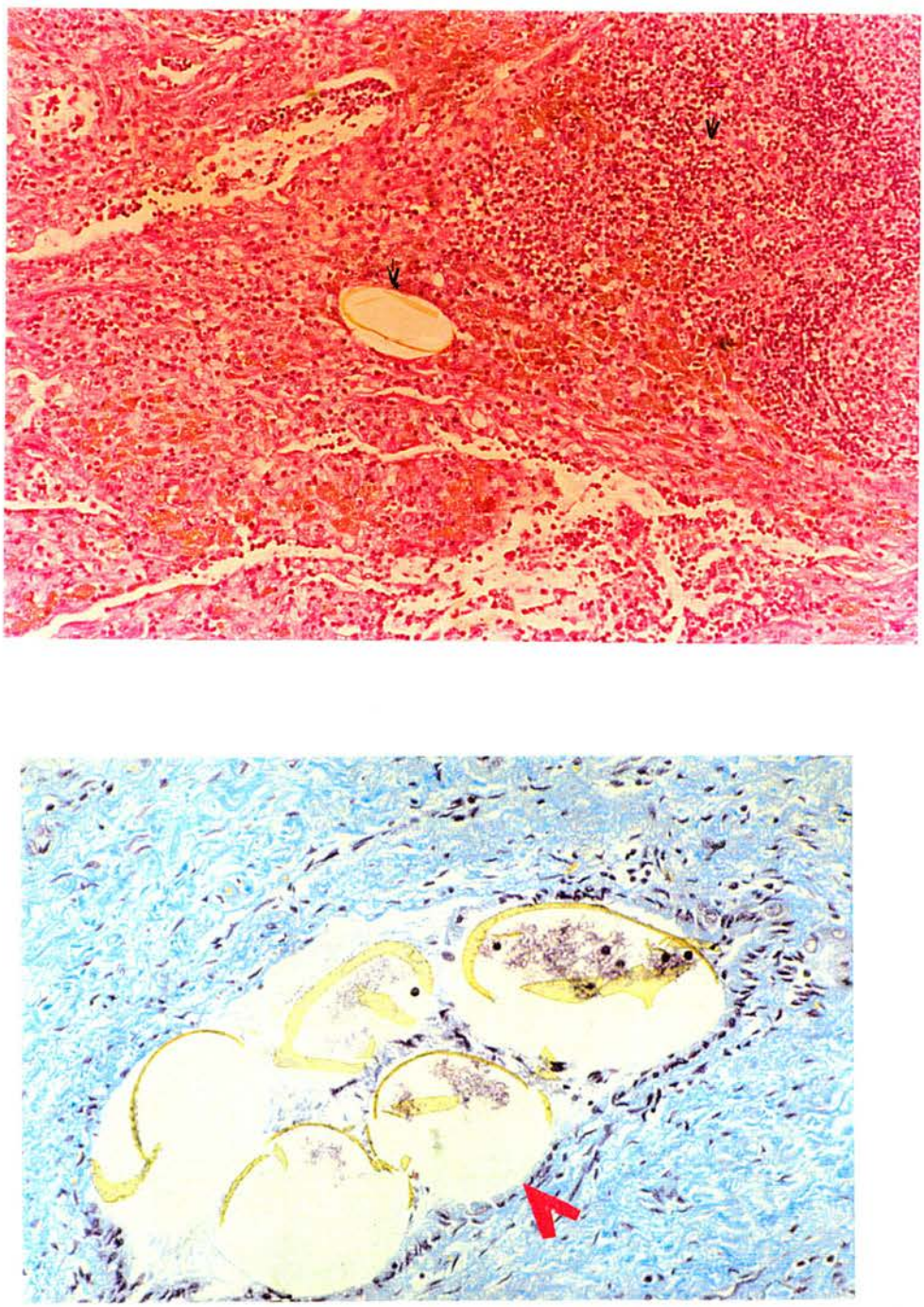


Figure 4.27: Photomicrographs of sections liver of calf 23c showing trapped fluke egg in the small bile ducts and severe necrosis with inflammatory cells infiltration (arrow) (H&E stained, x52 magnification) above. at the bottom is section of calf 23c liver showing trapped eggs in bile duct (arrow) (RMB stained, x52 magnification).

Haematology

Generally haematological evidence from the infected calves was consistent with light chronic infection . Calf 23c had a transient reduced packed cell volume (Figure 4.28) and also a decreased haemoglobin (Figure 4.28). There was no changes in MCV, MCH or values in any of the infected calves. (Figure 4.29 and Appendix Table 4.32 & 4.34).

White blood cell counts were very variable in infected calves but remained above that of the uninfected control calf 26c. Eosinophil values were highest in infected and challenged calf 23c (Figure 4.30. and Appendix Tables 4.35-4.37).

Clinical Biochemistry

The serum total protein and albumin values are presented in Appendix Table 4.38 and Figures 4.31 There was no apparent change in serum total protein and albumin values of all the animals.

The serum GLDH values in uninfected control calf 26 remained consistently low throughout the experiment (Figure 4.32a). However values of GLDH of infected cattle rose sharply by 2 wpi. peaking at 7 wpi. with values falling thereafter and reaching normal. There was a steady rise in serum Gamma Glutamyltransferase (γ-GT) activities in infected calves from 4 wpi., this reached the peak by 11 wpi. and fell thereafter to reach almost that of uninfected control calf by 21 wpi. (Figure 4.32b. and Appendix Table 4.4.39).

There was also no change in serum Glucose and β-Hydroxyl-Butyrate levels. This is consistent with the cattle having light infection (Appendix table 4.40 and Figures 4.33).

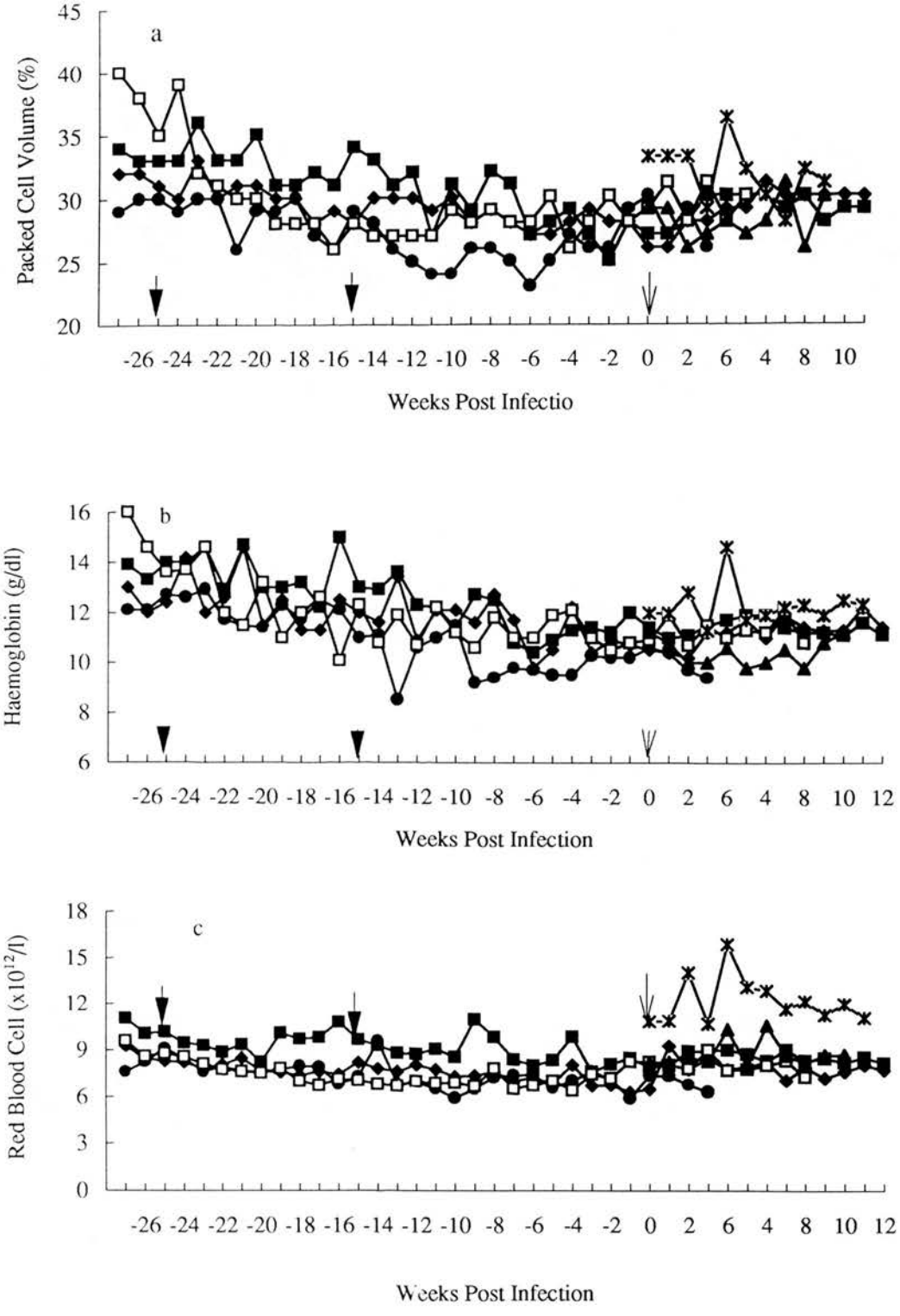


Figure 4.28: Pact Cell Volume (a) Haemoglobin (b) and red blood cell counts (c) in calves 14c, 34c and 45c with single *F. hepatica* (peruvian strain) infection, calves 15c and 23c with challenge infection and calf 26c uninfected control.
◄— Primary infection ⟵ Challenge infection

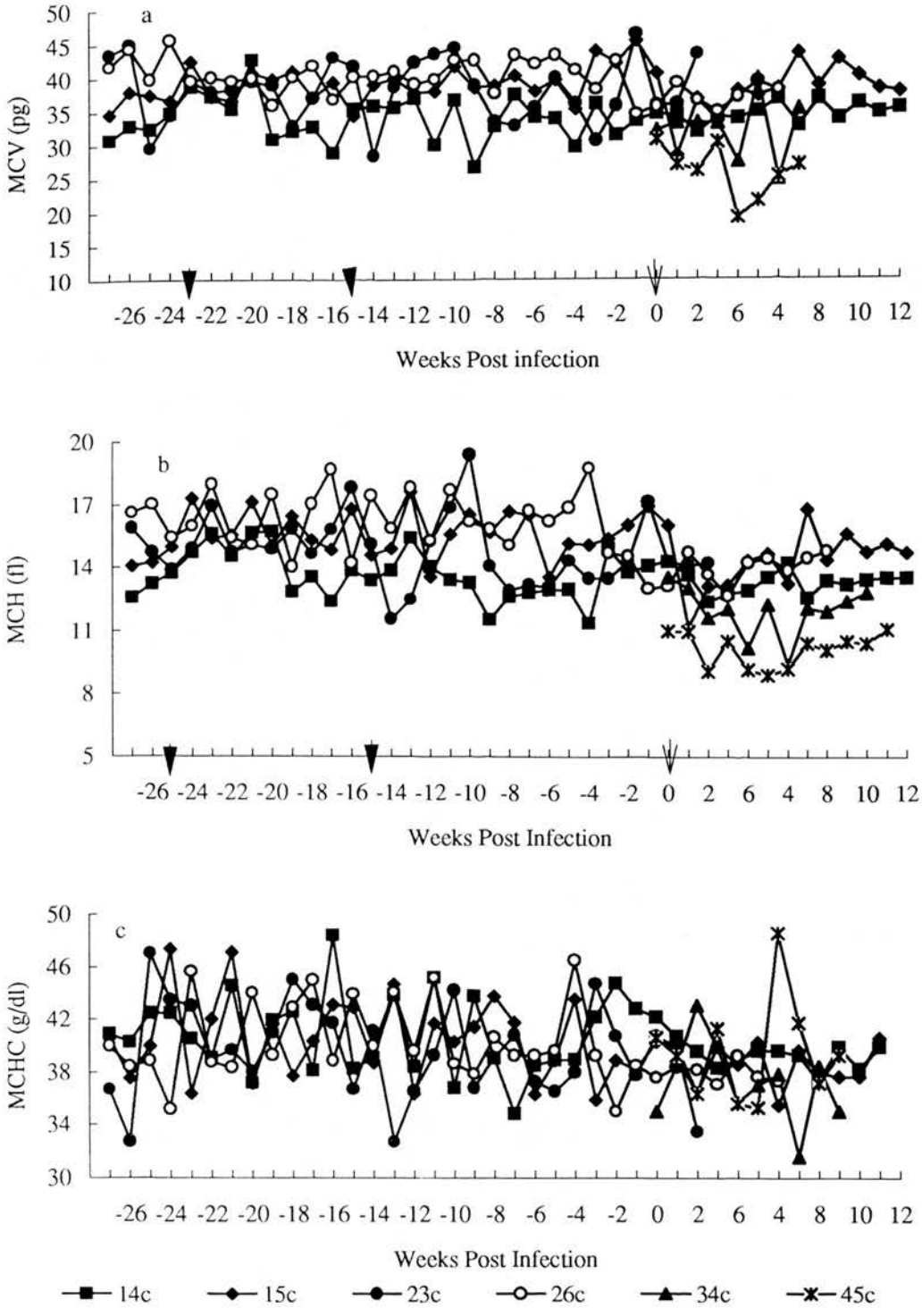


Figure 4.29: Mean Corpuscular Volume (MCV) (a) Mean Corpuscular Haemoglobin (MCH) (b) and Mean Corpuscular Haemoglobin Concentration (MCHC) (c) in calves14c, 34c and 45c withsingle F. hepatica (peruvian strain) infection, calves 15c and 23c with challenge infection and uninfected control calf 26

◄— Primary infection ⟵ Challenge infection

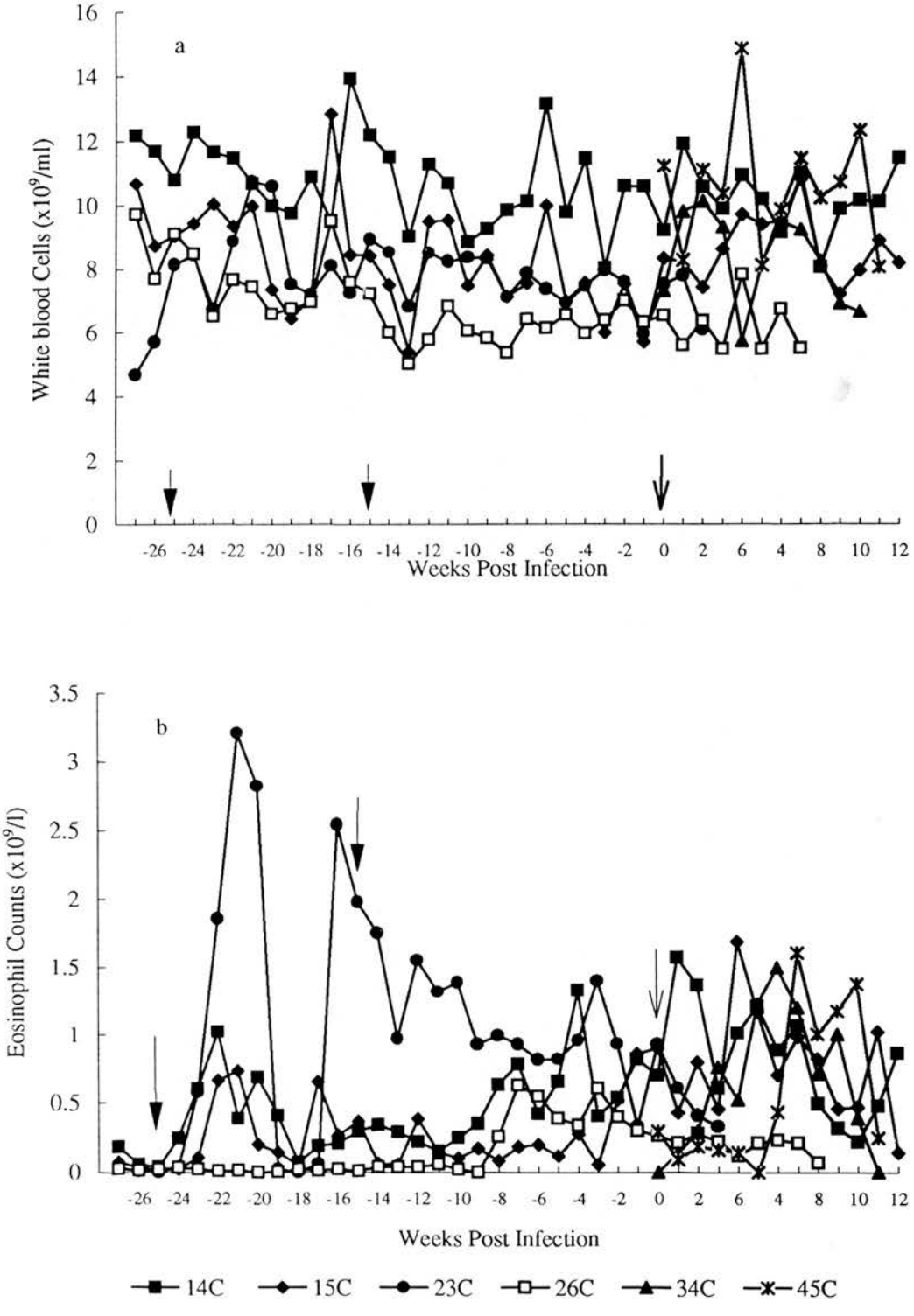


Figure 4.30: White Blood Cells (a) and Eosinophil counts (b) in Calves 14c, 34c and 45c with single *F. hepatica* (peruvian strain) infection Calves 15c and 23c with challenge infection and Calf 26c uninfected control.

◄— Primary infection ⟵ Challenge infection

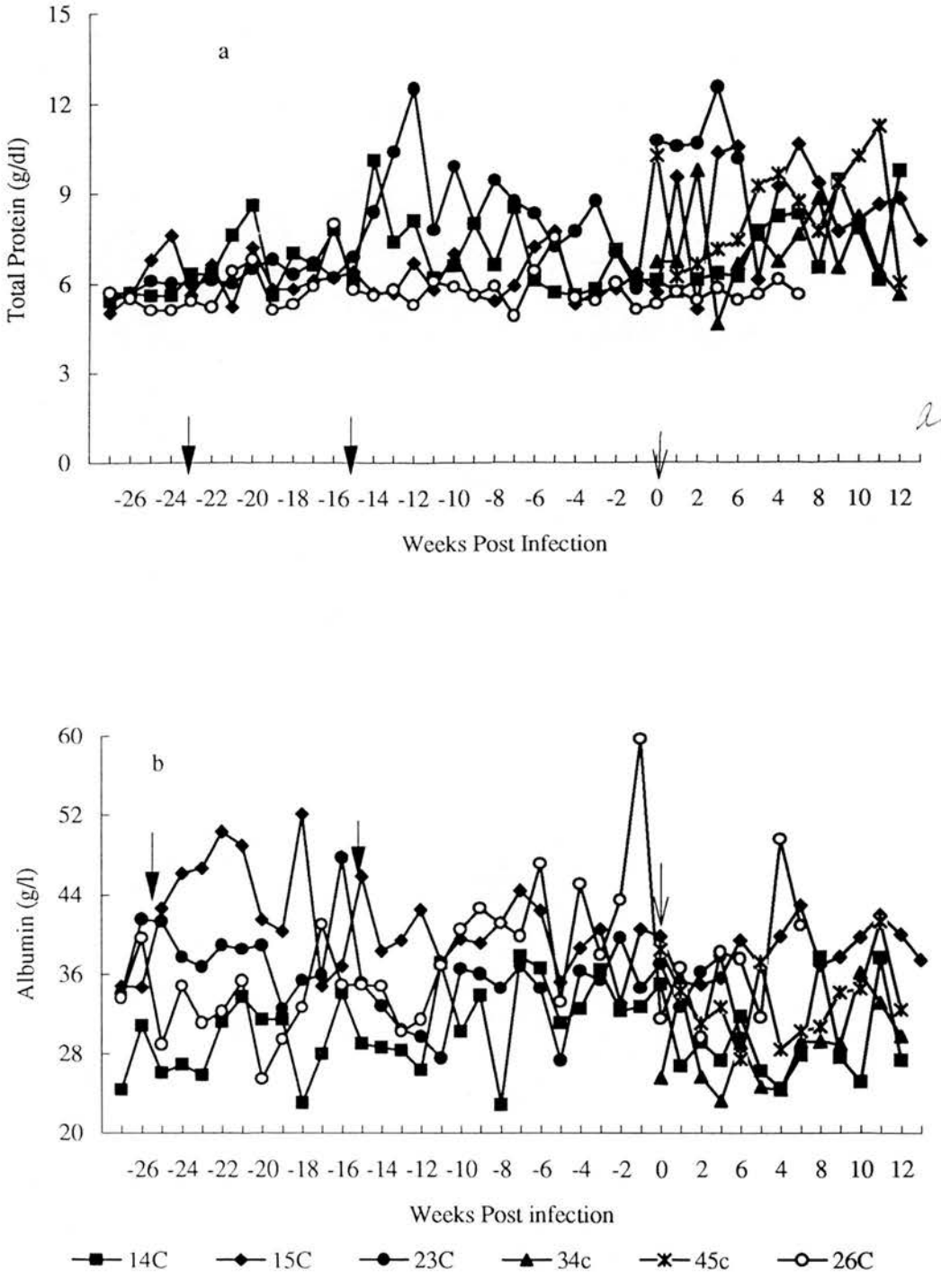


Figure 4.31: Albumin (a) and total Protein (b) values in calves14c, 34c and 45c with single *F. hepatica* (peruvian strain) infection, calves 15c and 23c with challenge infection and calf 26c as uninfected control

◄— Primary infection ←— Challenge infection

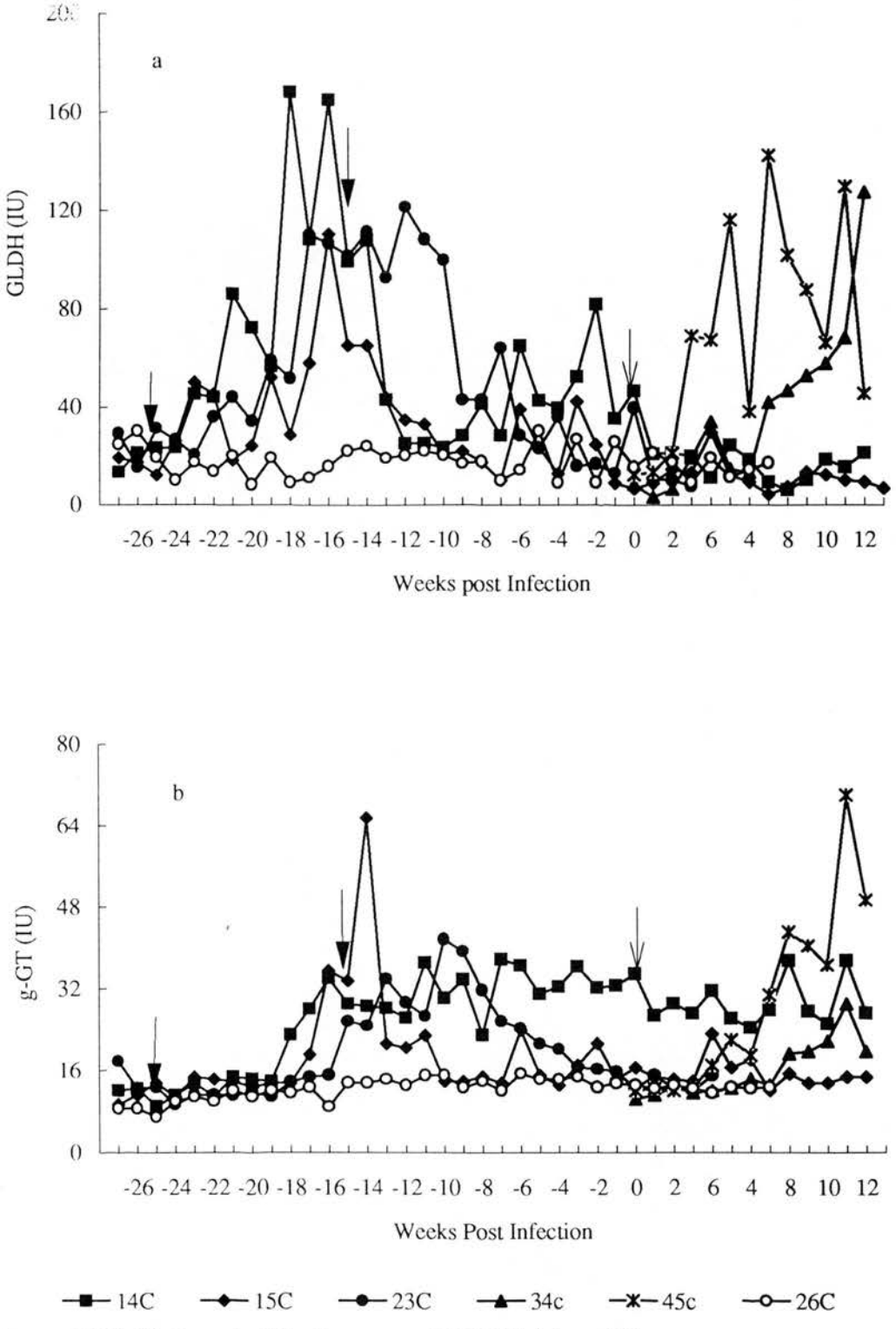
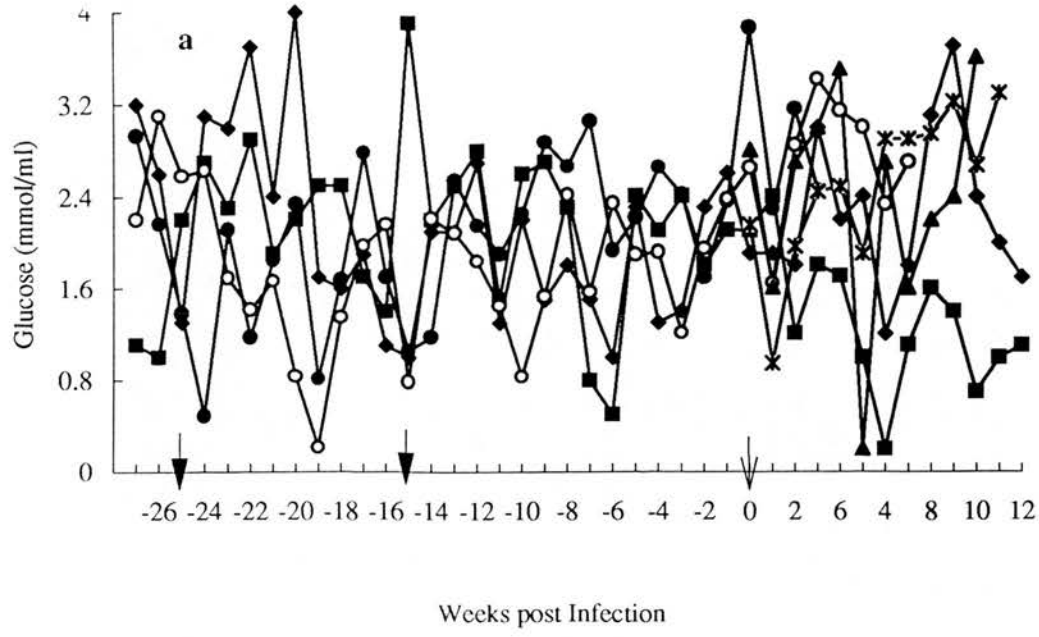


Figure 4.32: Glutamate dehydrogenase (GLDH) (a) and Gamma Glutamyltransferase (γ-GT) (b) values in calves 14c, 34cand 45c with single *F. hepatica* (peruvian strain) infection Calves 15c and 23c with challenge infection and Calf 26c uninfected control

◄— Primary infection ⟵ Challenge infection



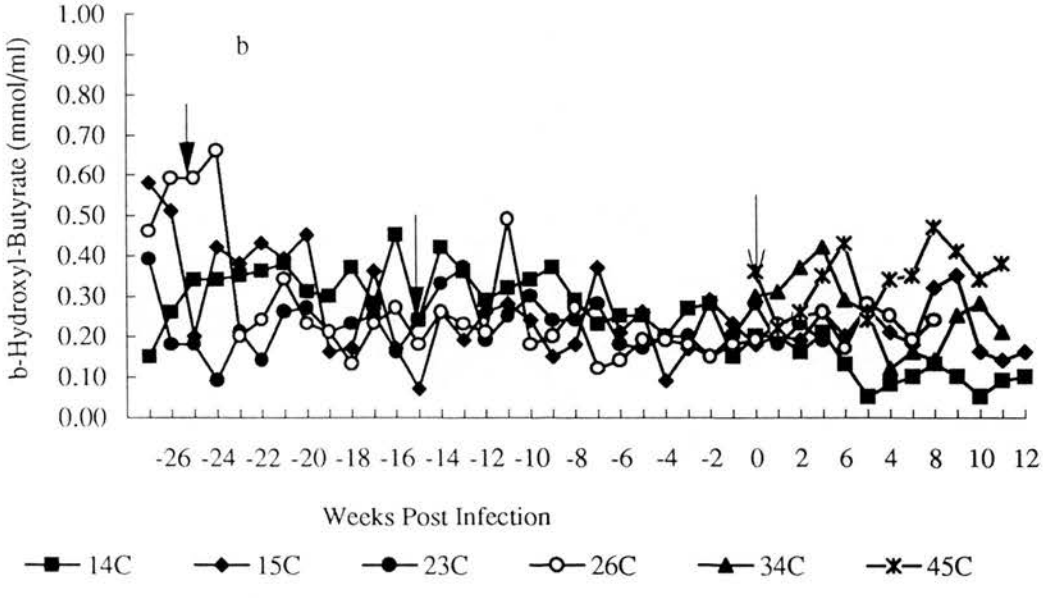


Figure 4.33: Glucose (a) and b-Hydroxyl-Butyrate (b) values in calves14c, 34c and 45c with single *F. hepatica* (peruvian strain) infection calves 15c and 23c with challenge infection and uninfected control calf 26c

◄— Primary infection ⟵ Challenge infection

4.1.6 Experiment 6: *F. Gigantica* (Kenyan strain) infection in Cattle

The general monitoring including clinical examination, parasitology and pathology, of this group was carried out by Nyanzunda (1993).

Parasitology

As judged by faecal egg counts, none of the cattle were infected with nematodes at any point in the monitoring period. Infected calves reached patency 14 wpi. (Table 4.6). The fluke recoveries were very low (Table.4.7) with calf 24 having the lowest. The fluke recoveries in all the infected calves were rather low at <8%.

Table 4.6: Experiment 6: Faecal egg count details of calves infected with 400 *F. gigantica* (Kenyan strain) from Nyanzunda (1993).

Weeks Post Infection	Calf 22	Calf 23	Calf 24	Calf 26
10	0	0	0	0
11	0	0	0	0
12	0	0	0	0
13	0	0	0	0
14	6	7	5	0
17	6	37	10	0
24	8	29	13	0

Table 4.7: Experiment 6: Some parasitological details of Calves infected with 400 *F. gigantica* (Kenyan strain) from Nyanzunda (1993).

Procedure	Calf No.	Prepatency (Weeks)	Flukes No	Flukes %
Infection	22	9	30	7.5
	23	11	31	7.75
	24	9	10	2.5
Uninfected	26	-	-	-

Pathology

No abnormalities in the size or consistency of the 3 calves' livers were observed. Major lesions were in the biliary system. The bile ducts were thickened and the epithelium of the common bileduct and gall bladder showed areas of haemorrhage and inflammation. The hepatic lymph nodes were hypertrophied. Upon subjective comparison of the extent of the lesions calf 23 had most pathological changes followed by calf 22 and calf 24 had the least pathological changes. This is consistent with the fluke recoveries, calf 23 had the highest fluke burden and calf 24 had the least as shown in section.

Haematology

Very few haematological factors were evident in the infected cattle, which was consistent with a mild chronic *F. gigantica* infection. Although there was no change in RBC and PVC levels (Figure 4.34), there was a noticeable drop in haemoglobin levels 7-18 wpi. (Figure 4.35a). There were no apparent changes in MCV, MCH or MCHC (Figure 4.35c & 36).

Although the results were very mild, there was a clear rise in WBC and eosinophil counts in the infected cattle. Although lymphocyte count in the infected cattle was raised (Figure 4.37) there were no apparent changes in any other cells in the WBC series. Haematological data is in appendix tables 4.41-4.44.

Clinical Biochemistry

There was no severe change in serum total protein and albumin values of all the animals. The results of Glucose and β-Hydroxybutyrate are shown in Appendix Tables 4.45 and Figures. 4.38.

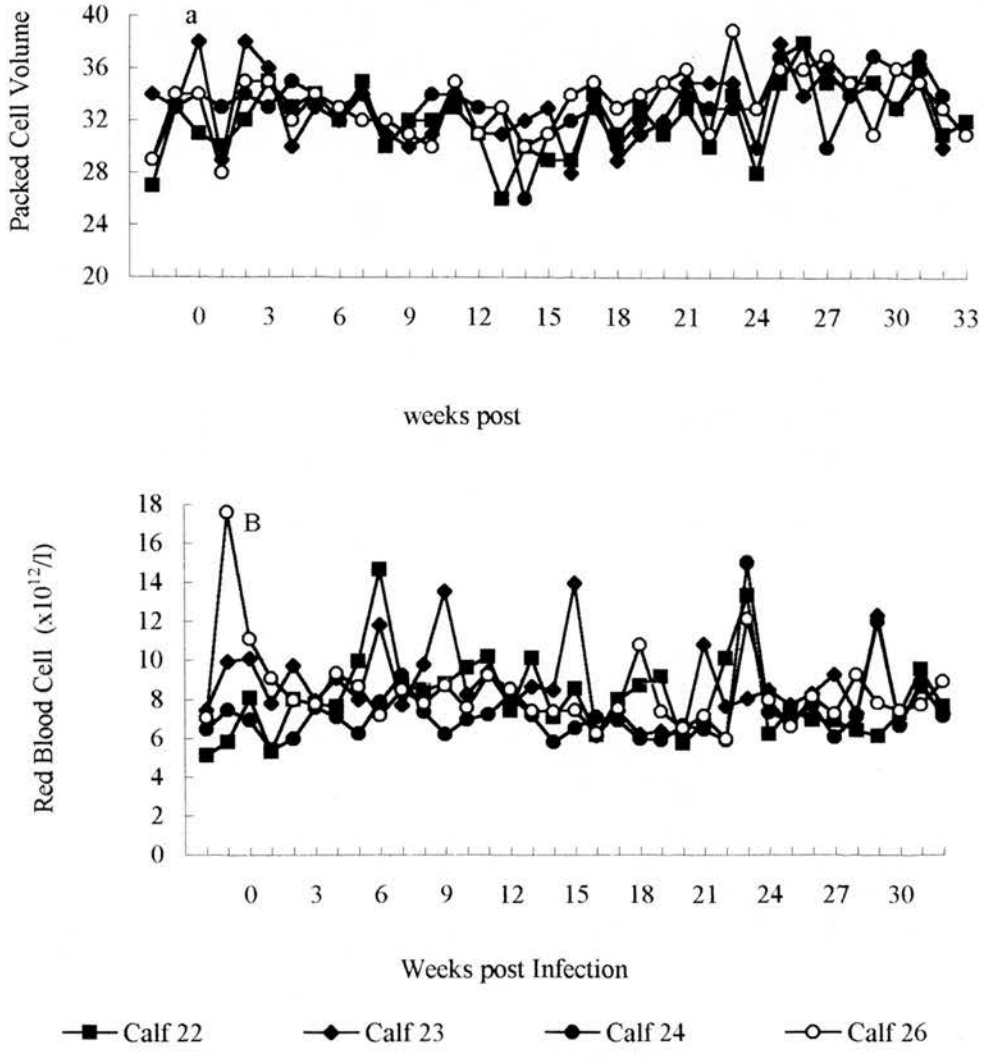


Figure 4.34: Red Blood Cell Counts (a) and Packed Cell Volume (b) of calves 22, 23 and 24 infected with 400 *F. gigantica* metacercariae (Kenyan strain) and uninfected control calf 26.

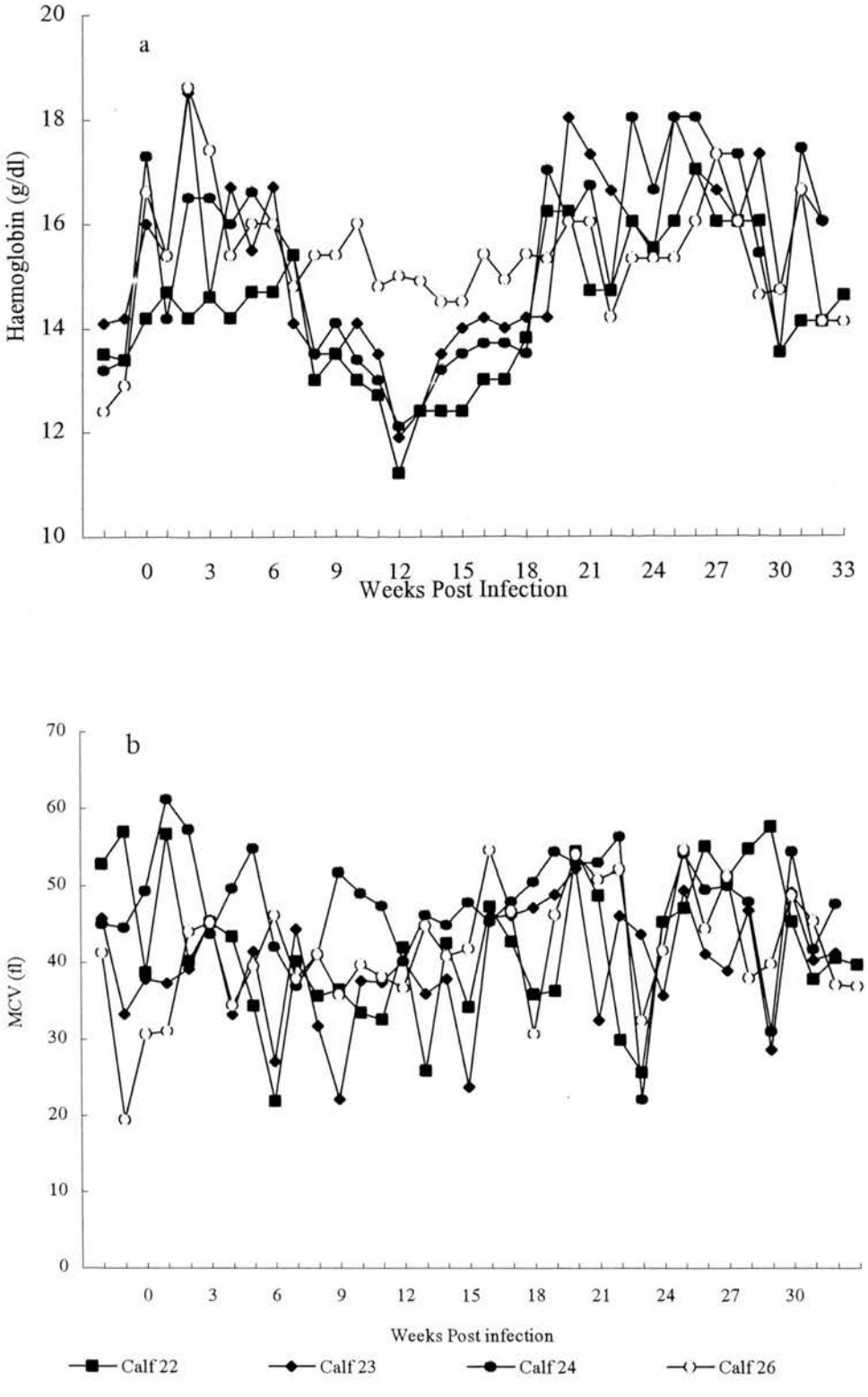


Figure 4.35: Haemoglobin (a) and MCV (b) of calves 22, 23 and 24 infected with 400 *F. gigantica* metacercariae (Kenyan strain) and uninfected control calf 26.

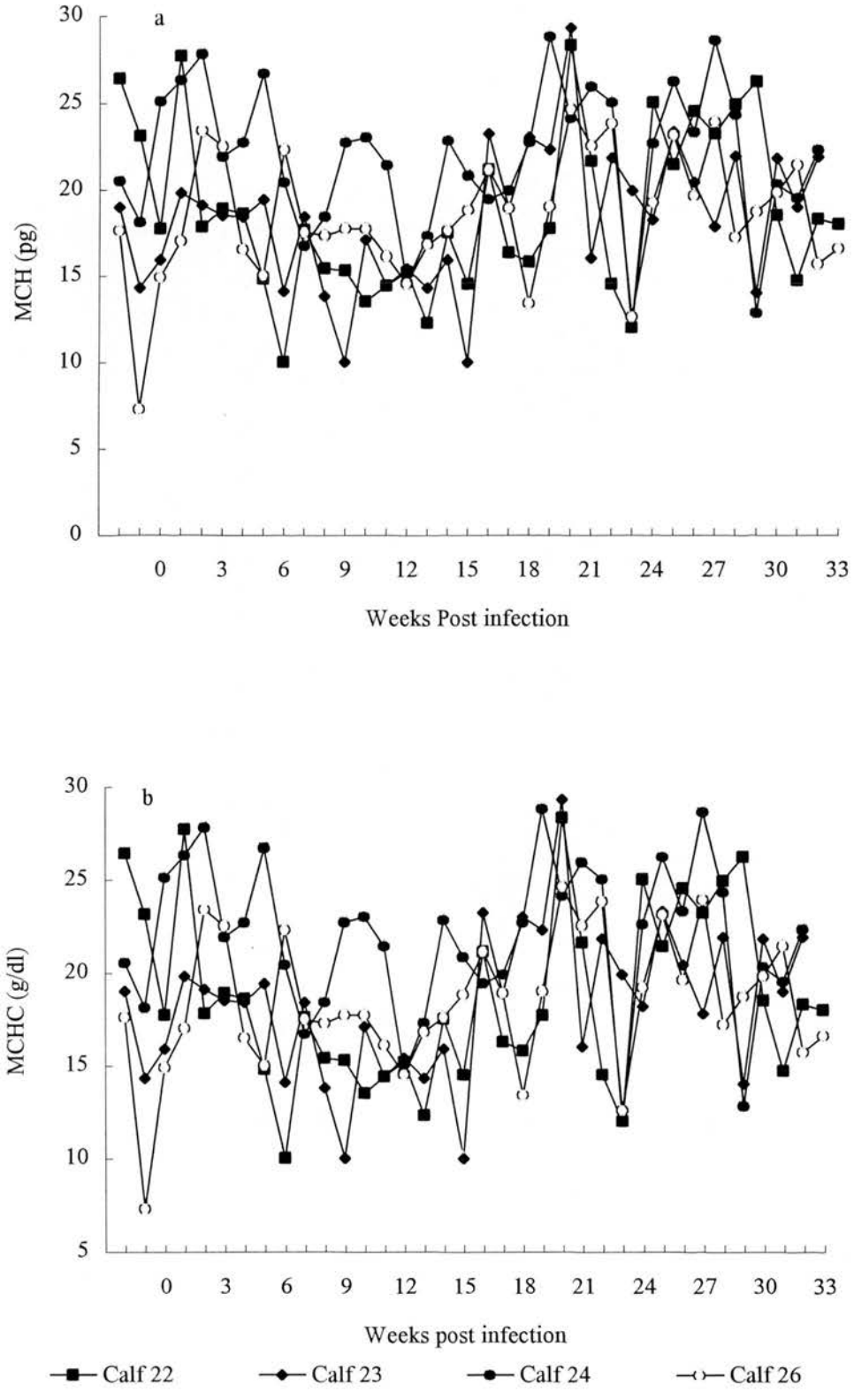


Figure 4.36: MCH (a) and MCHC (b) of calves 22, 23 and 24 infected with 400 *F. gigantica* metacercariae (Kenyan strain) and uninfected control calf 26.

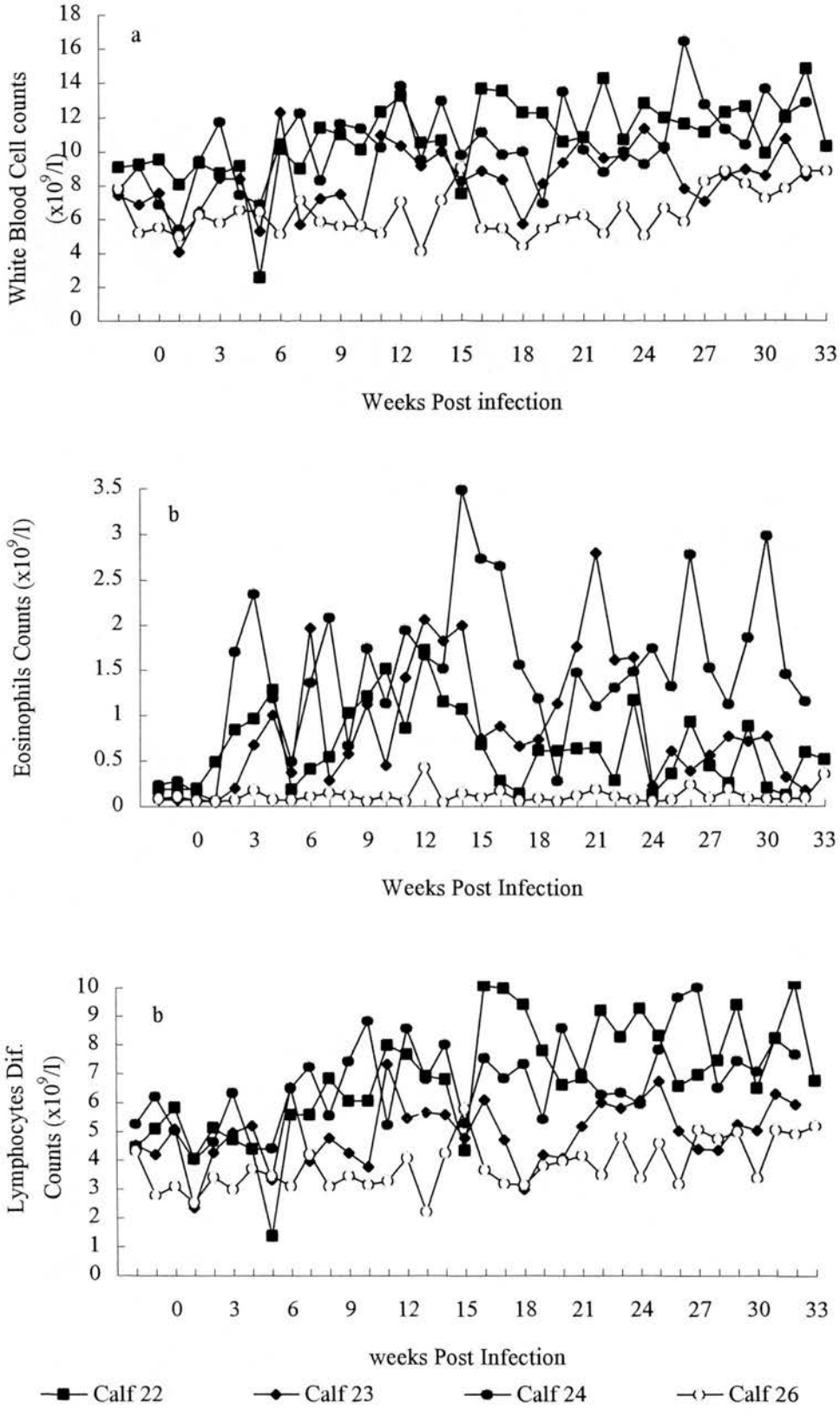


Figure 4.37: White Blood Cell Counts (a)and Eosinophils Counts (b) and Lymphocytes (c)Counts (x109/l) of calves 22, 23 and 24 infected with 400 *F. gigantica* metacercariae (Kenyan strain) and uninfected control calf 26.

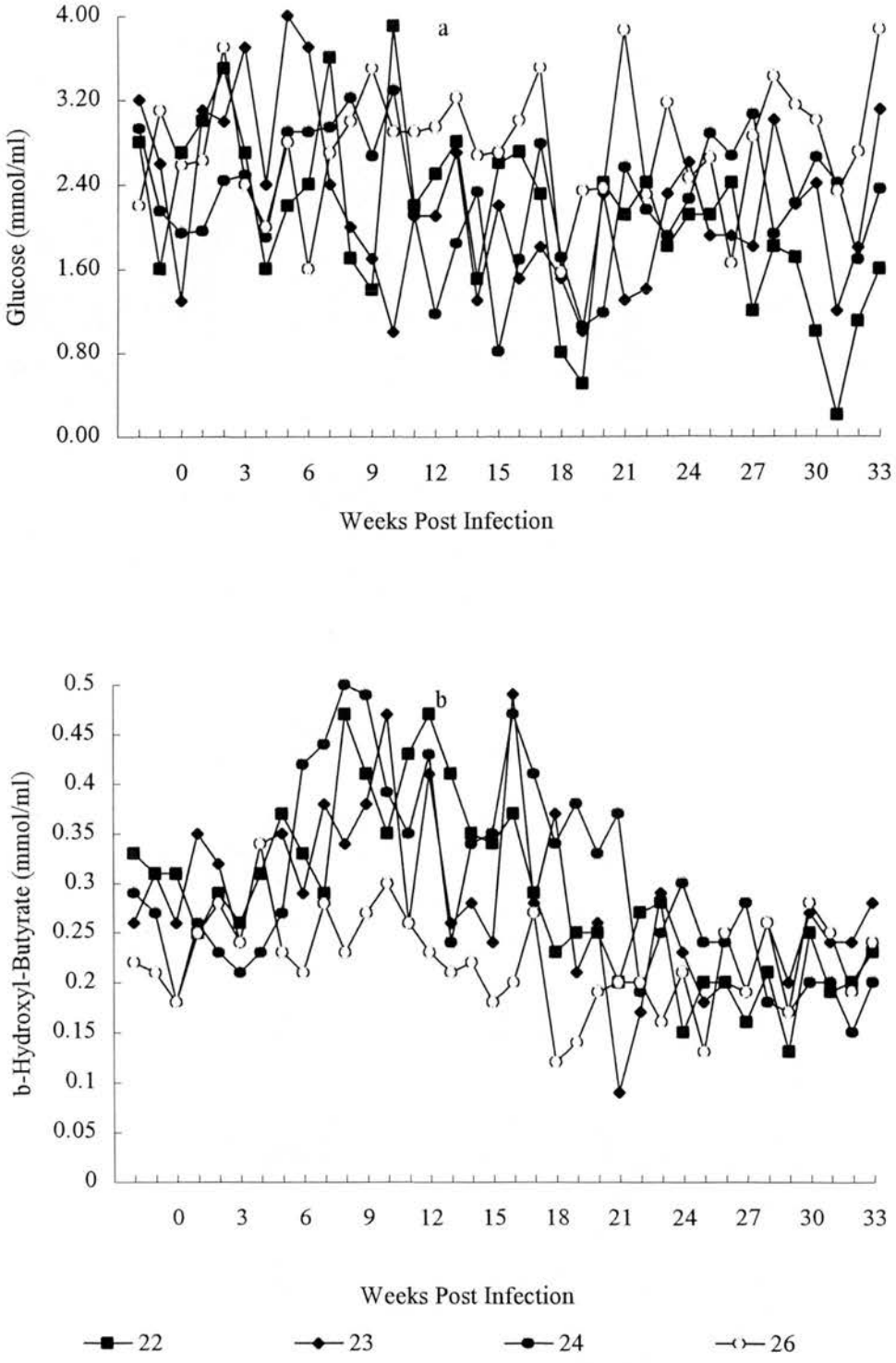


Figure 4.38: Glucose (a) and b-Hydroxyl-Butyrate (b) of calves 22, 23 and 24 infected with 400 *F. gigantica* metacercariae (Kenyan strain) and uninfected control calf 26.

4.2 SERUM ANTIBODY RESPONSES OF SHEEP AND CATTLE TO EXCRETORY/SECRETORY PRODUCTS OF *FASCIOLA* SPP

4.2.1 Determination of Optimum Assay Conditions by Titration

The optimum assay conditions i.e. those which optimised the signal to the background ratios, were determined by titration of antigen i.e. either *F. hepatica* (Fh-E/S) or *F. gigantica* (Fg-E/S) E/S products), serum and conjugate for the polyclonal detection system or antigen (Fh-E/S or Fg-E/S), serum, monoclonal antibody and conjugate for the monoclonal antibody based detection systems. Figures 4.39-4.46 show the titration's for total Ig and IgG_1 in both *F. hepatica* and *F. gigantica* infected sheep and cattle representing the polyclonal total Ig and the monoclonal antibody (IgG_1) based detection systems.

Chequerboard titration for serum, monoclonal antibodies and conjugate were carried out by diluting these systems in blocking buffer using doubling serial dilution ranging from 20-0.313µg/ml (antigen), 1:50-1:1600 (serum), 1:20-1:320 (monoclonal antibody) and 1:1000-1:32,000 (conjugate). Titrations were run in duplicate and the mean values calculated. The chosen antigen concentration, serum, monoclonal antibody and conjugate dilution were used in all subsequent sequential screenings. The two positive sera P1 and P2 were taken from *F. hepatica* or *F. gigantica* infected animals and corresponded to 8, 9 or 10 wpi (P1) and 21, 22 or 23 wpi (P2) for sheep and 7 wpi (P1) and 32 wpi (P2) for calves. These times post infection were chosen to assess responses at the middle and at the end of the experimental period. The selected concentrations for all the assays, total Ig, IgG_1, IgG2, IgM and IgA for sheep and cattle are in Table 4.8-4.10. See Appendix Table 4.46-4.55 for full details.

Table 4.8: Sheep infected with either *F. hepatica* or *F. gigantica*: Optimal dilution of antigen (μg/ml), serum and conjugate for polyclonal antibody system using Excretory/Secretory products from *F. hepatica* and *F. gigantica* as antigen (Fh-E/S and Fg-E/S)

Assay	*F. hepatica*			*F. gigantica*		
	Fh-E/S (μg/ml)	Serum	Conj.	Fg-E/S (μg/ml)	Serum	Conj.
Total Ig	1.25	1:400	1:4000	1.25	1:200	1:4000
IgM	2.5	1:200	1:2000	2.5	1:200	1:2000

Table 4.9 Sheep infected with *F. hepatica* or *F. gigantica*: optimal dilution of antigen (μg/ml), serum, monoclonal antibody (McAb)and conjugate for monoclonal antibody system using excretory/secretory products from *F. hepatica* and *F. gigantica* as antigen (Fh-E/S and Fg-E/S)

Assay	*F. hepatica*				*F. gigantica*			
	Fh-E/S (μg/ml)	Serum	McAb	Conj.	Fg-E/S (μg/ml)	Serum	McAb	Conj.
IgG_1	1.25	1:400	1:40	1:4000	1.25	200	1:40	1:4000
IgG_2	5	1:50	1:20	1:1000	5	1:50	1:20	1:1000
IgA	5	1:50	1:20	1:1000	5	1:50	1:20	1:1000

Table 4.10: Cattle infected with *F. hepatica* or *F. gigantica*: Optimal dilution of antigen (μg/ml), serum and conjugate for polyclonal antibody detection systems using Excretory/Secretory products from *F. hepatica* and *F. gigantica* (Fh-E/S and Fg-E/S) as antigen.

Assay	*F. hepatica*			*F. gigantica*		
	Fh-E/S (μg/ml)	Serum	Conj.	Fg-E/S (μg/ml)	Serum	Conj.
Total Ig	1.25	1:200	1:4000	1.25	1:200	1:4000
IgG_1	1.25	1:200	1:4000	1.25	1:200	1:4000
IgM	2.5	1:200	1:2000	2.5	1:200	1:4000
IgG_2	2.5	1:50	1:1000	2.5	1:50	1:1000
IgA	2.5	1:50	1:1000	2.5	1:50	1:1000

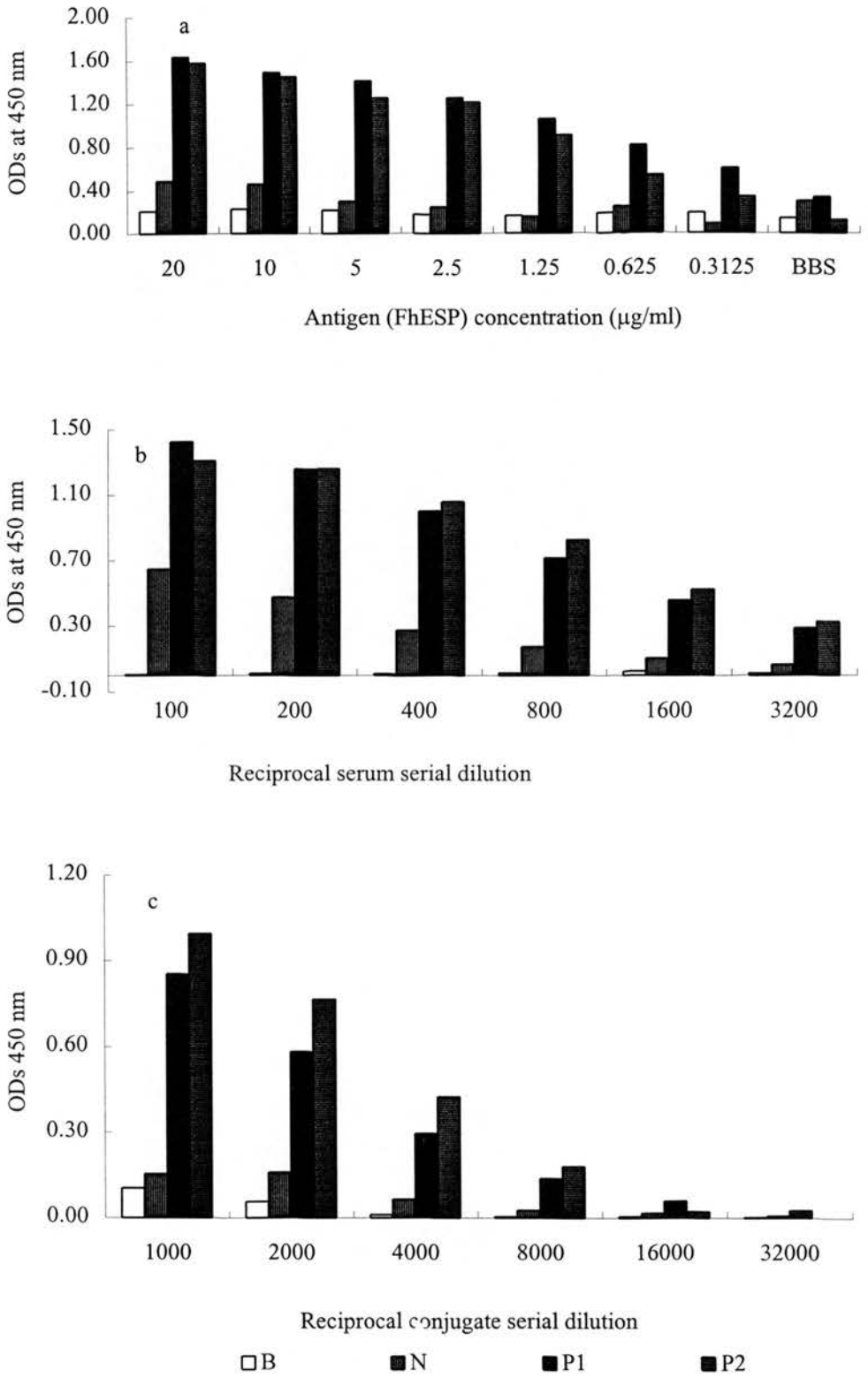


Figure 4.39: Antigen (Fh-E/S) (a), serum (b), and conjugate (c) titrations for total Ig for *F. hepatica* infected and uninfected control sheep showing the mean ELISA OD (450 nm) values obtained for diluent (B) negative (N) positive (P1) and positive (P2)

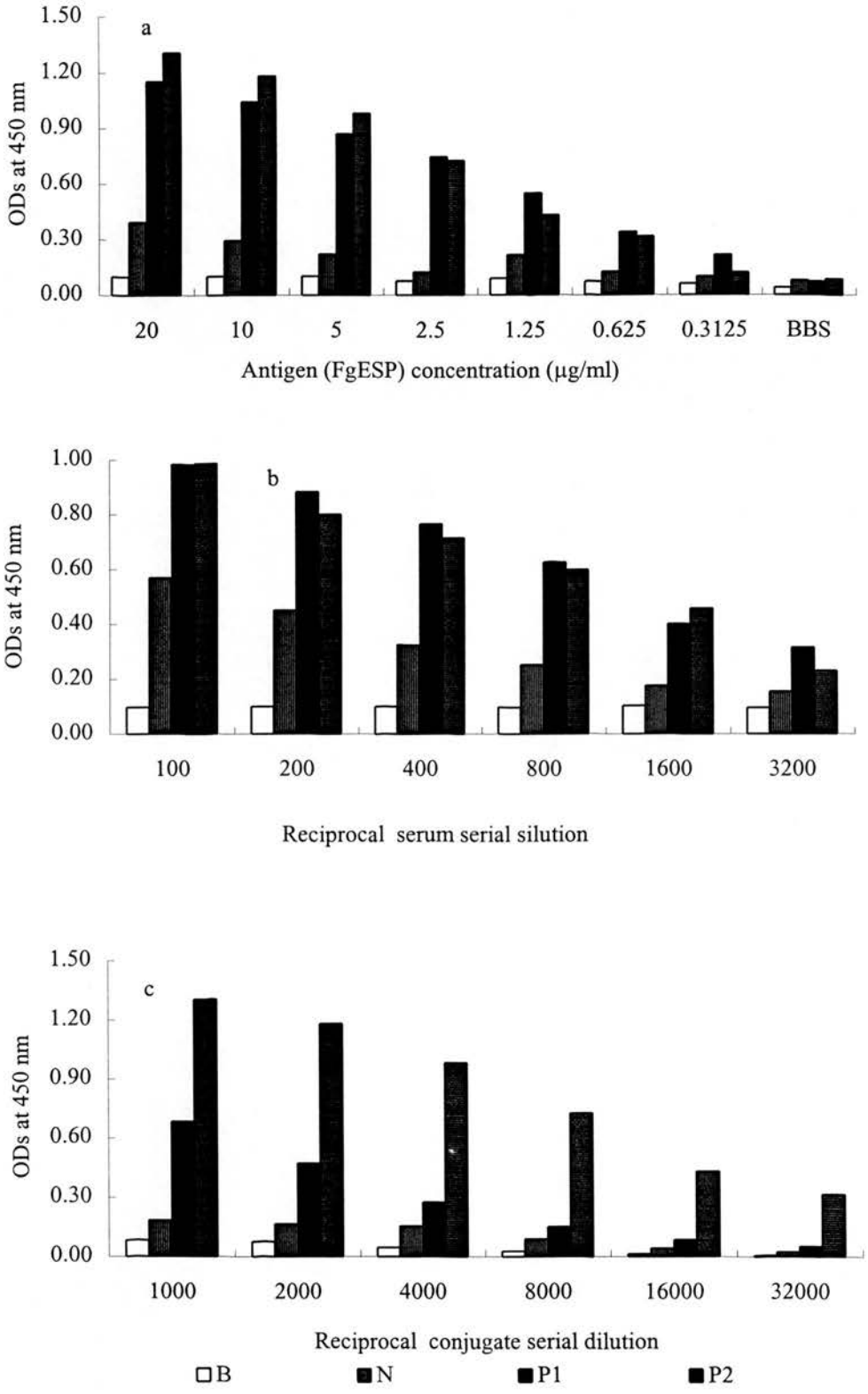


Figure 4.40: Antigen (Fg-E/S) (a), serum (b), and conjugate (c) total Ig for *F. gigantica* infected and uninfected control sheep showing titrations for the mean ELISA OD (450 nm) values obtained for diluent (B), negative (N), positive (P1) and positive (P2)

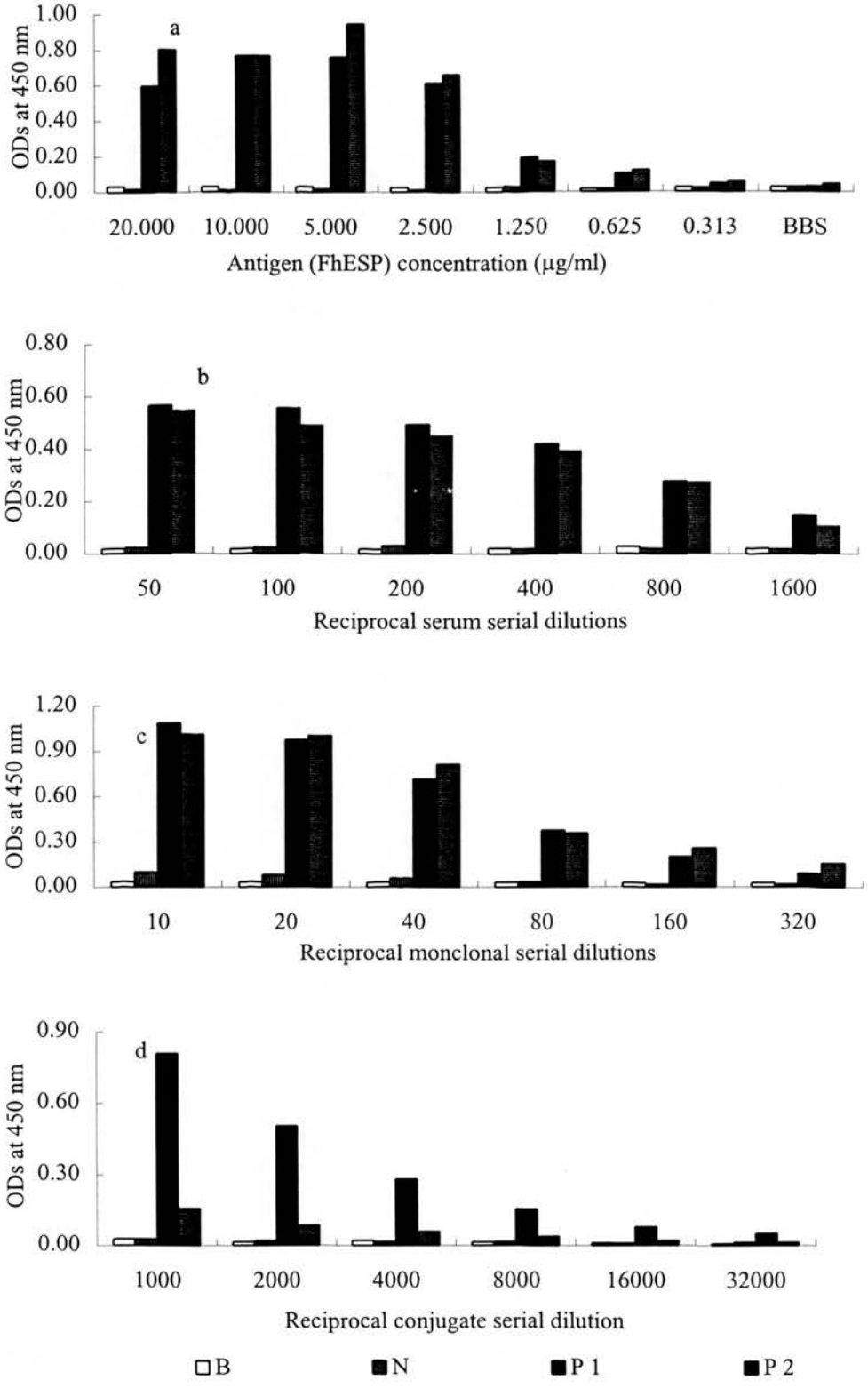


Figure 4.41: Antigen (Fh-E/S) (a), serum (b), monoclonal (c) and conjugate (d) titrations for IgG_1 for *F. hepatica* infected and uninfected control sheep showing the mean ELISA (450 nm) values obtained for diluent (B) negative (N) positive (P1) and positive (P2)

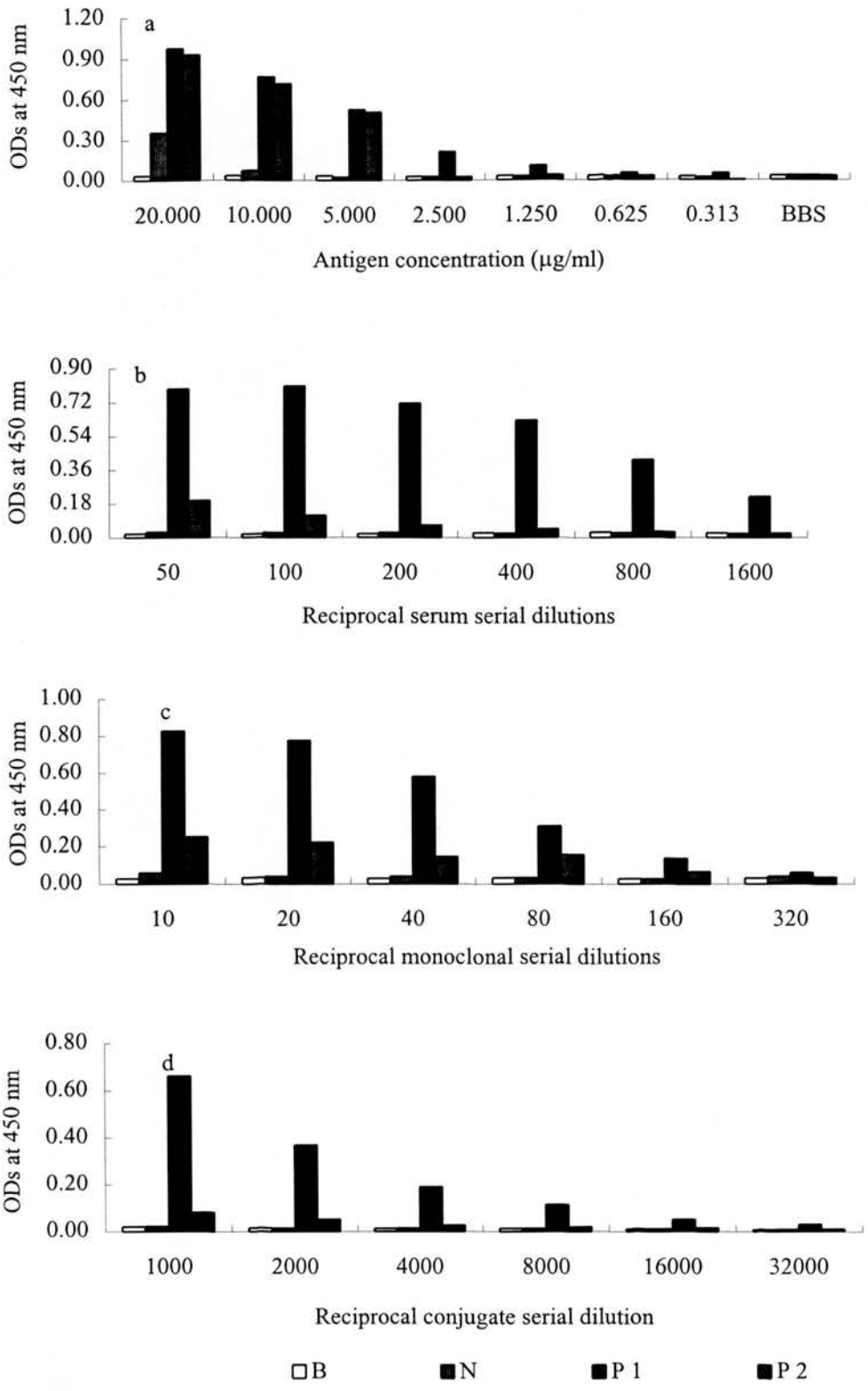


Figure 4.42: Antigen (Fg-E/S) (a), serum (b), monoclonal (c) and conjugate (d) titrations for IgG_1 for *F. gigantica* infected and uninfected control sheep showing the mean ELISA (450 nm) values obtained for diluent (B) negative (N) positive (P1) andpositive (P2)

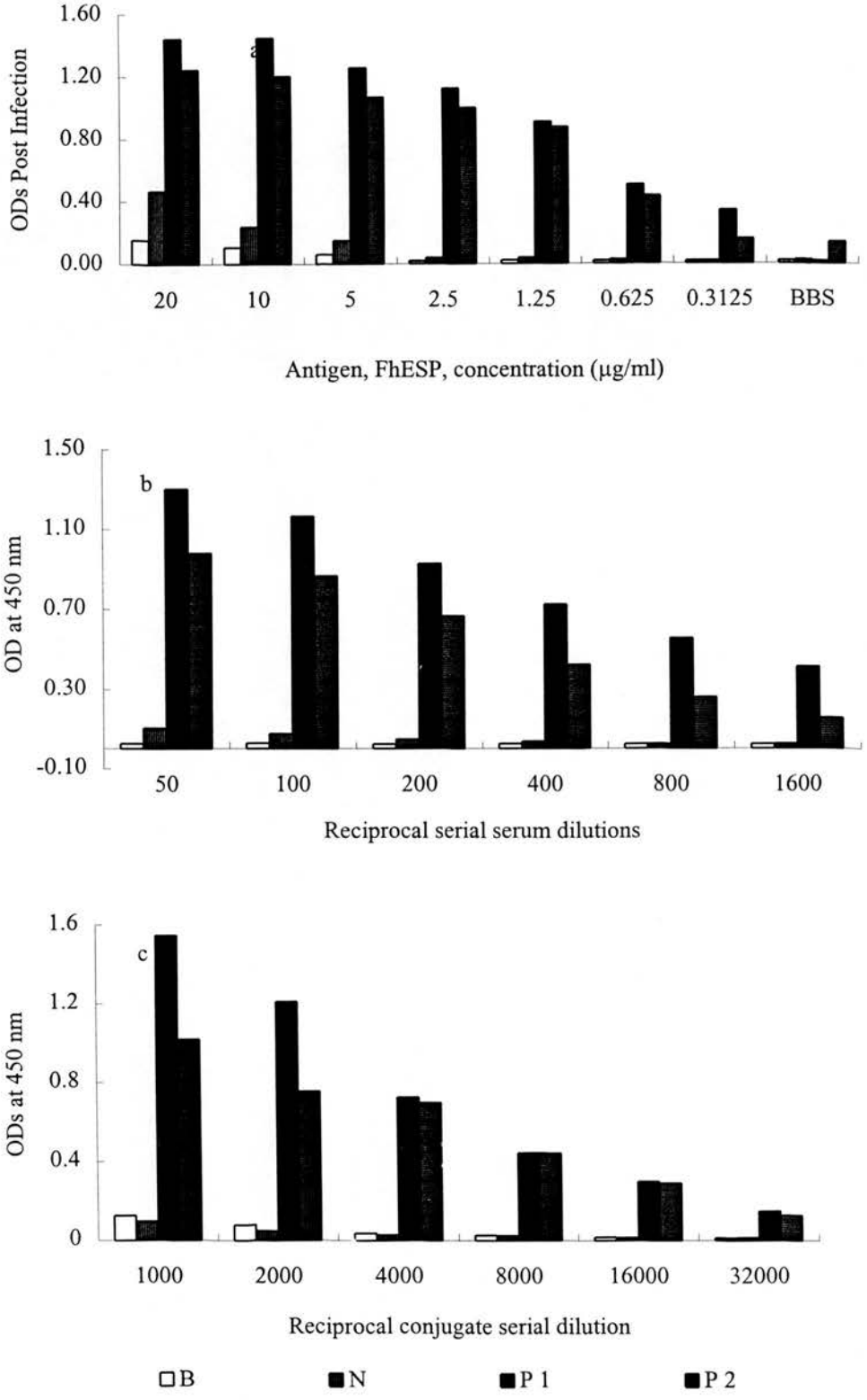


Figure 4.43: Antigen (Fh-E/S) (a), serum (b), and conjugate (c) titrations for total Ig for *F. hepatica* infected calves and uninfected control calves showing the mean ELISA (450nm) values obtained for diluent (B), negative (N), positive (P1) and positive (P2)

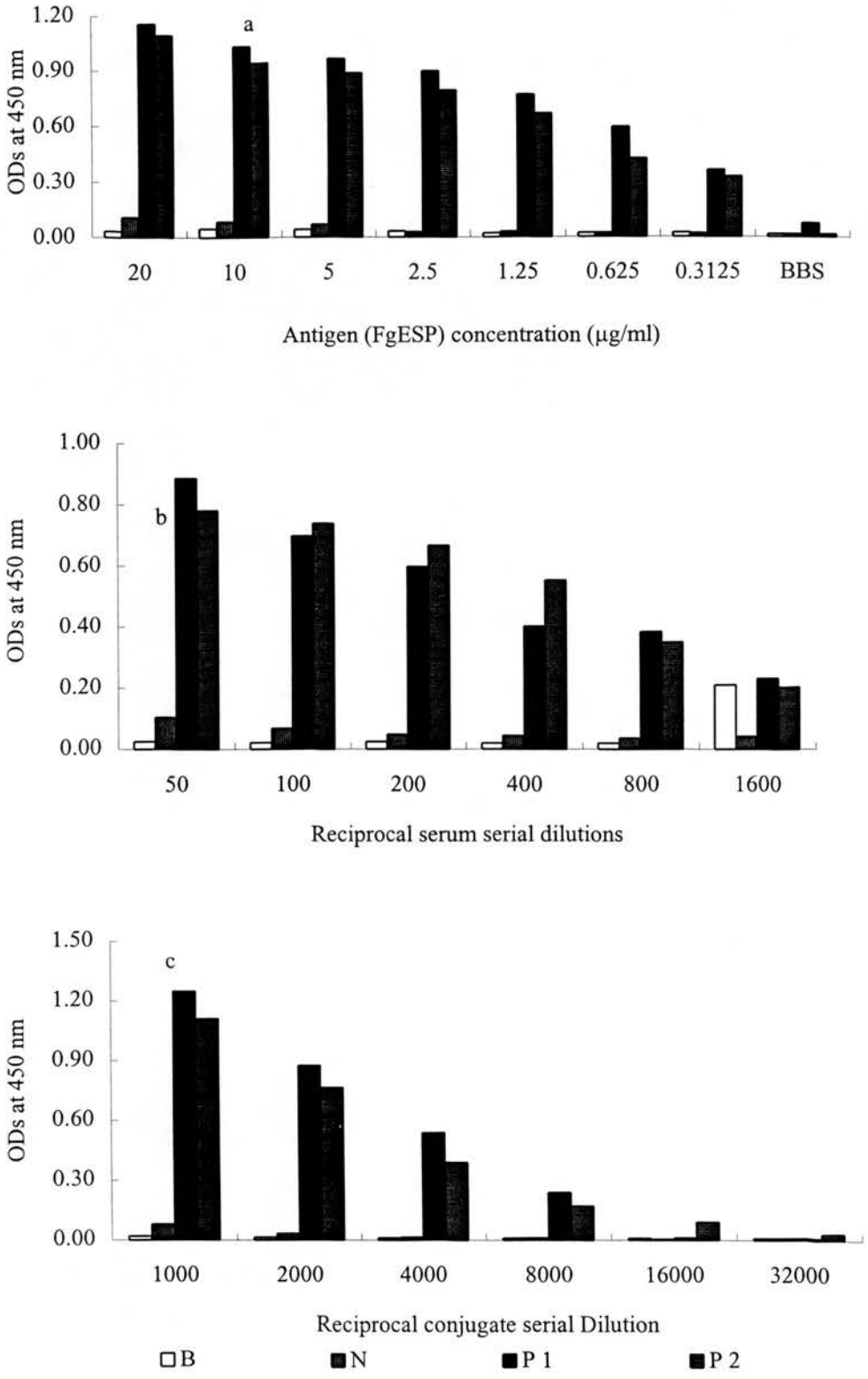


Figure 4.44: Antigen (Fg-E/S) (a), serum (b), and conjugate (c) titrations for total Ig for *F. gigantica* infected calves and uninfected control calves showing the mean ELISA (450 nm)values obtained for diluent (B), negative (N), positive (P1) and positive (P2)

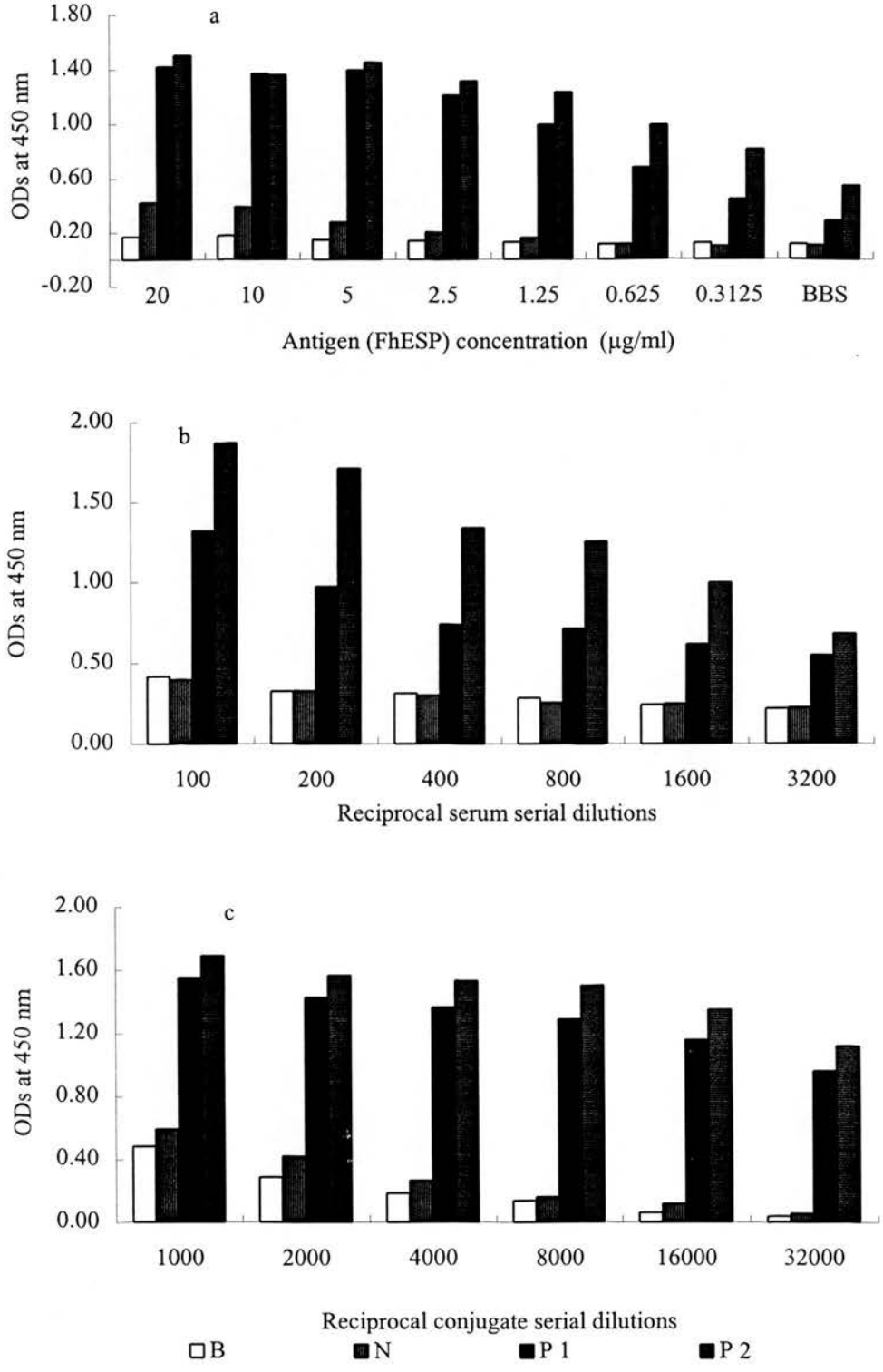


Figure 4.45 Antigen (Fh-E/S) (a), serum (b), and conjugate (c) titrations for IgG_1 for *F. hepatica* infected calves and uninfected control calves showing the mean ELISA (450 nm) values obtained for diluent (B), negative (N), positive (P1) and positive (P2)

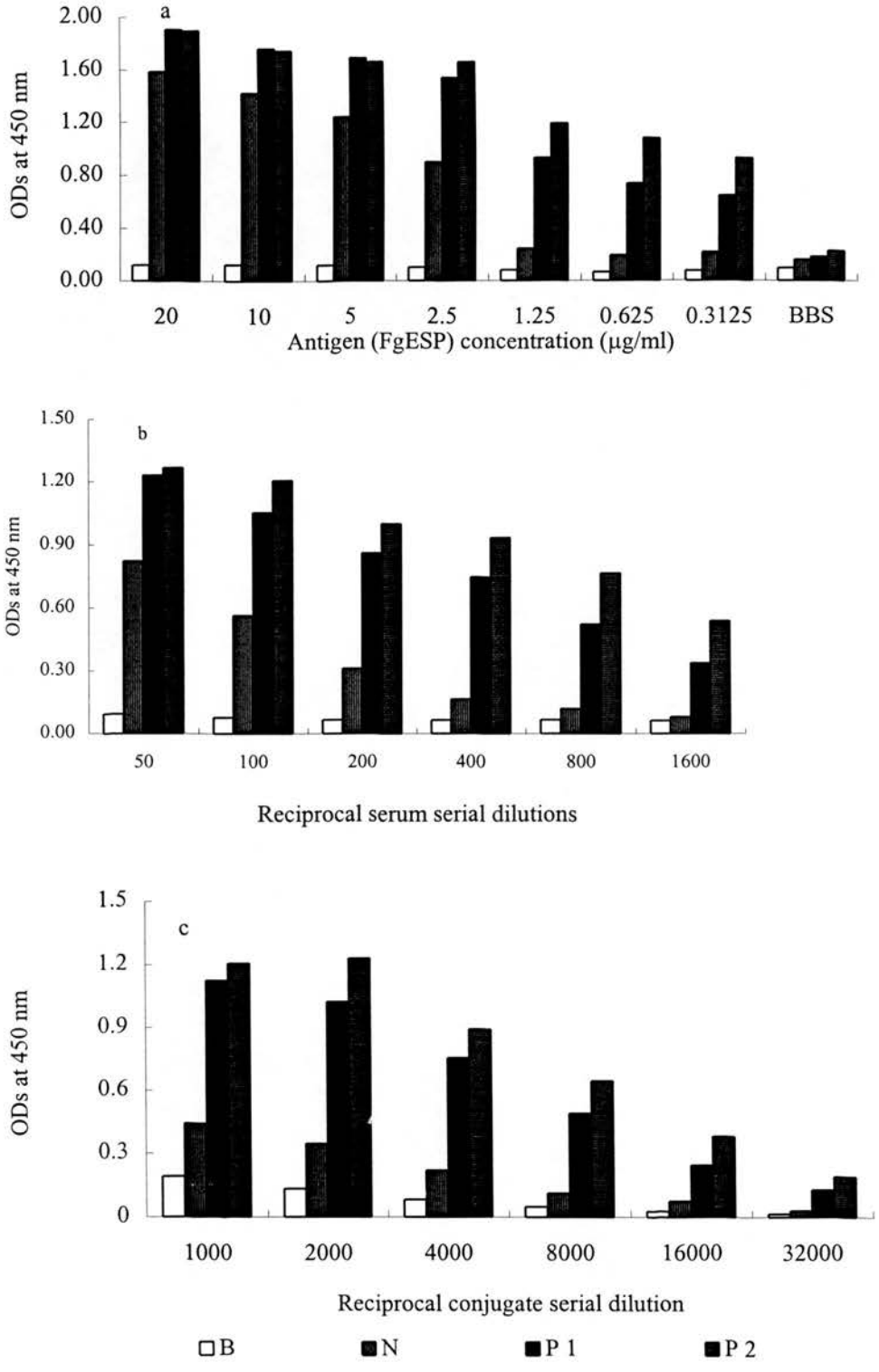


Figure 4.46: Antigen (Fg-E/S) (a), serum (b), and conjugate (c) titrations for IgG_1 for *F. gigantica* infected calves and uninfected control calves showing the mean ELISA (450 nm) values obtained for diluent (B), negative (N), positive (P1) and positive (P2)

4.2.2 Experiment 1: *F. hepatica* (Peruvian and British strain) Infection in Sheep

Following primary infection, total Ig, IgG_1, IgM, IgG_2 and IgA antibody responses to Fh-E/S were easily detected in the infected sheep but IgG_2 responses were relatively poor hence the Y-axis scale is different from the rest of the figures (Figures 4.47a-4.48b)

All the infected sheep showed an increase in total Ig levels from 2 week post infection (wpi.) and peak OD values in sheep 9 and 10 were obtained at 10 wpi. These levels were high throughout experimental period. However the antibody response by sheep 5 began to fall by 10 wpi. and it died at 12 wpi.

IgG_1 isotype responses to Fh-E/S were strongest in sheep 9 and 10 from 1-17 wpi. The IgG_1 antibody response was first detected in the rest of the infected sheep +2 wpi. (Figures 4.47b). IgM levels were clearly noticeable in all the infected sheep. Responses were recorded from 2 wpi. peaking 6 wpi. but declining by 14 wpi. One of the *F. hepatica* infected sheep (7) showed low IgM responses than the rest of infected in this group (Figures 4.47c). IgG_2 levels in infected sheep particularly in sheep 6 and 7 infected with 300 *F. hepatica* metacercariae were clearly higher than the uninfected control (Figures 4.48a). IgA response to Fh-E/S appeared biphasic a sharp increase in IgA response to Fh-E/S was noticed in all the *F. hepatica* infected sheep by +2 wpi. dropping by +4 wpi. IgA levels started to rise very slightly in all the infected sheep 14 wpi., this was however less so in sheep 10 (Figures 4.48b). The adjusted data in appendix 4.56-4.58

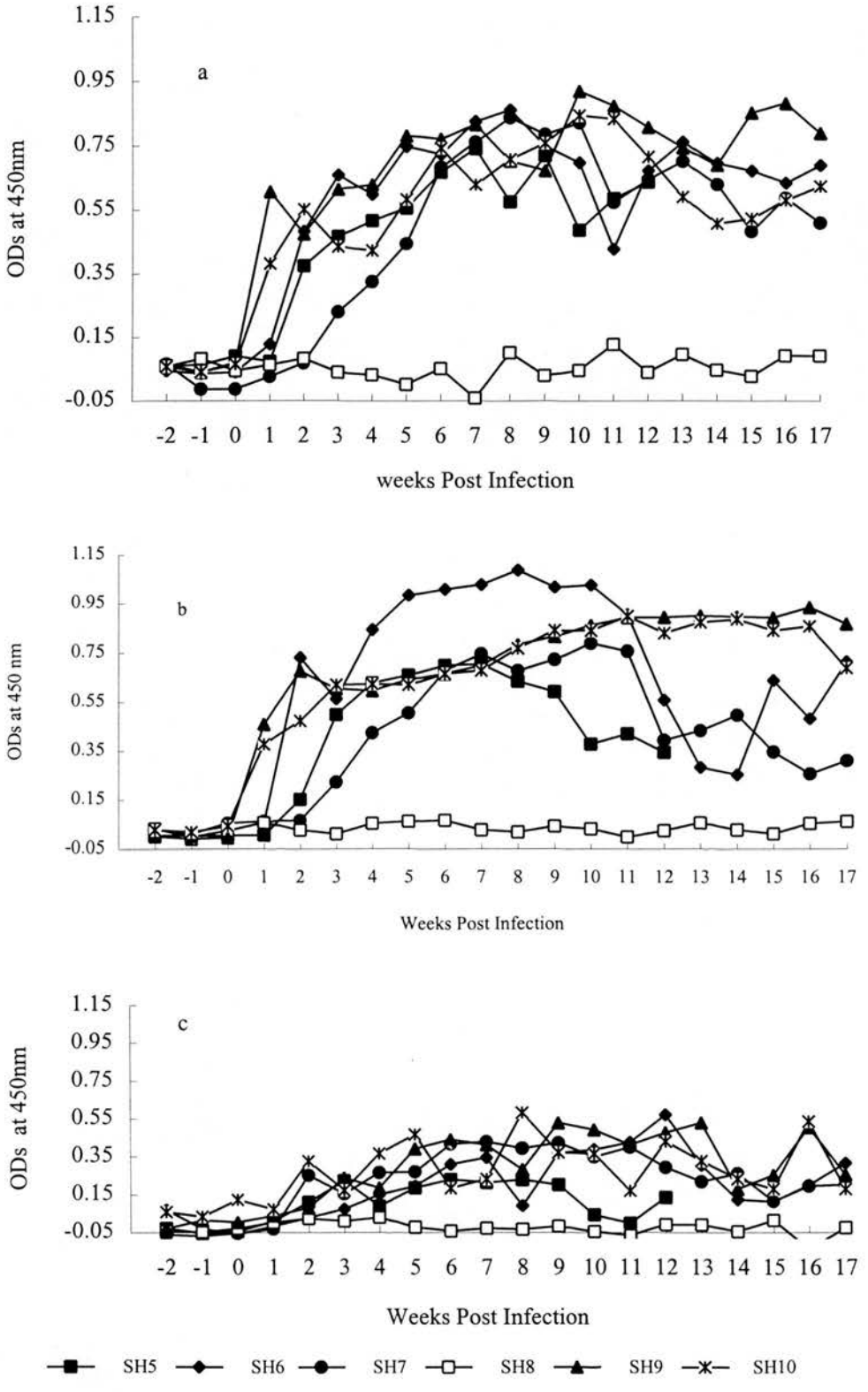


Figure 4.47: The adjusted ELISA OD (450 nm) for total Ig (a), IgG1 (b) of *F. hepatica* infected and IgM (c) responses sheep (5, 6, 7, 9 and 10) and uninfected control sheep (8) to FhESP

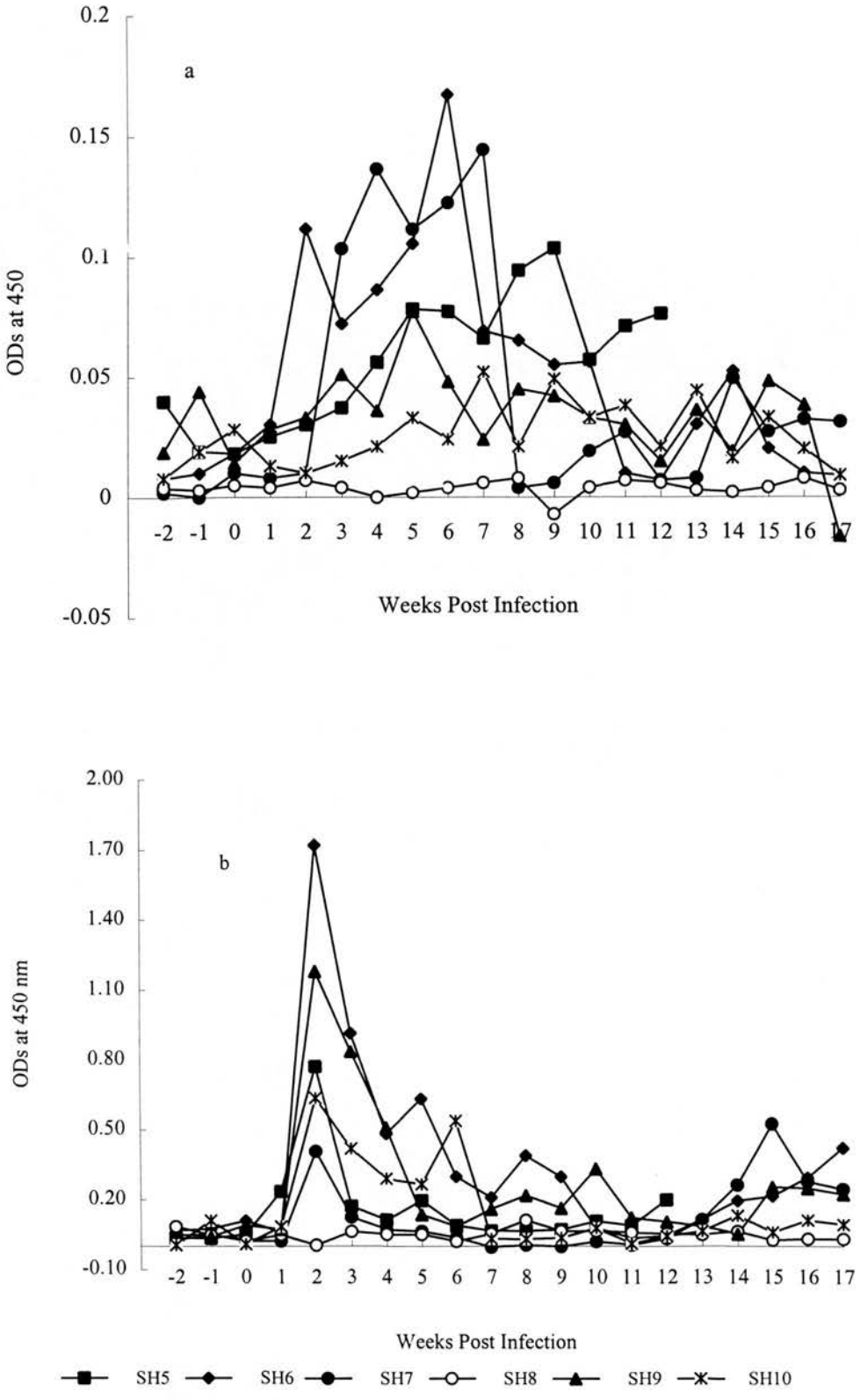


Figure 4.48: The adjusted ELISA OD (450 nm) values of IgG2 (a) and IgA (b) responses of *F. hepatica* infected sheep (5, 6, 7, 9 and 10) and uninfected control sheep (8) to FhESP

4.2.3 Experiment 2: *F. hepatica* (British strain) Infection in Sheep

These sheep developed more pronounced antibody response than those in experiment 1 (Figures 49a-50b). The adjusted data for each sheep are presented in Appendix Tables 4.59-61.

Antibody to Fh-E/S were easily detected in *F. hepatica* infected sheep. The total Ig IgG_1 and IgM responses were more pronounced while IgG_2 and IgA were the lowest.

The infected sheep showed increased total Ig levels +4 wpi. peaking at 10-15 wpi. Although the levels remained high throughout the experiment there was a clear reduction in response by +22 wpi. (Figure 4.49a). IgG_1 isotype responses were first detected by +6 wpi. peaking at 12-16 wpi. and remained high up to the end of experiment (Figure 4.49b). IgM responses were first detected by +2 wpi. peaking at 4-8 wpi., showing a progressive decrease thereafter. (Figure 4.49c).

IgG_2 levels increased sharply starting from 2 wpi. Sheep 24 showed the strongest response peaking at 8 wpi. (Figure 4.50a) and decrease by 11-12 wpi. Apart from Sheep 24 IgG_2 levels in infected sheep remained slightly above uninfected animals up to 22 wpi.

A sharp increase in IgA response to Fh-E/S was noticed in all the *F. hepatica* infected animals. IgA showed a clear biphasic response at +2-8 wpi. and 16-22 wpi. As with the previous experiment the second phase was very short so that by the end of the experiment (22 wpi.) the ODs had reduced to just above those of the uninfected control sheep (Figure 4.50b).

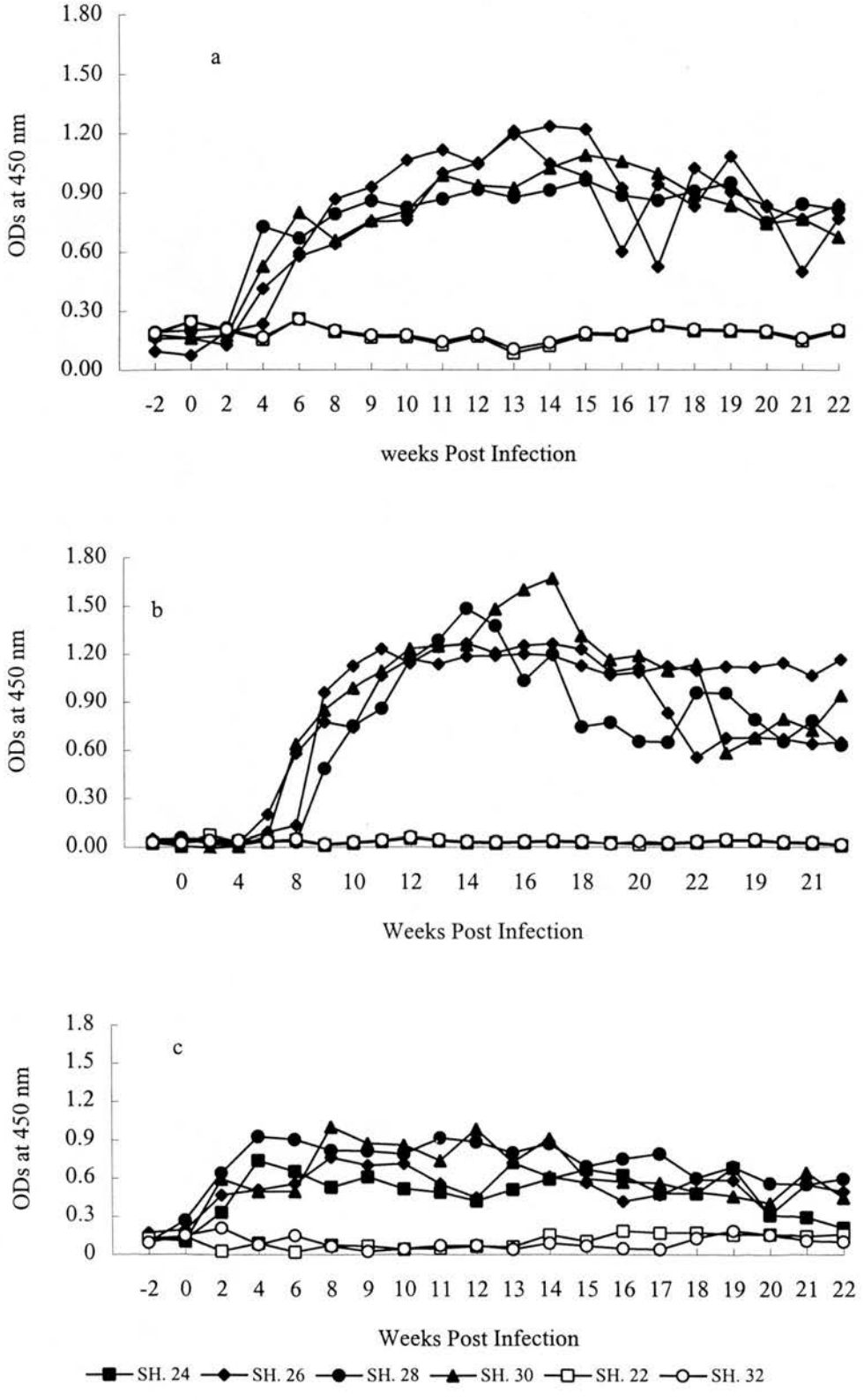


Figure 4.49: The adjusted ELISA OD (450 nm) for total Ig (a), IgG1 (b) and IgM (c) responses of *F. hepatica* infected sheep (24, 26, 28 and 30) and uninfected control sheep (22 and 32) to FhESP

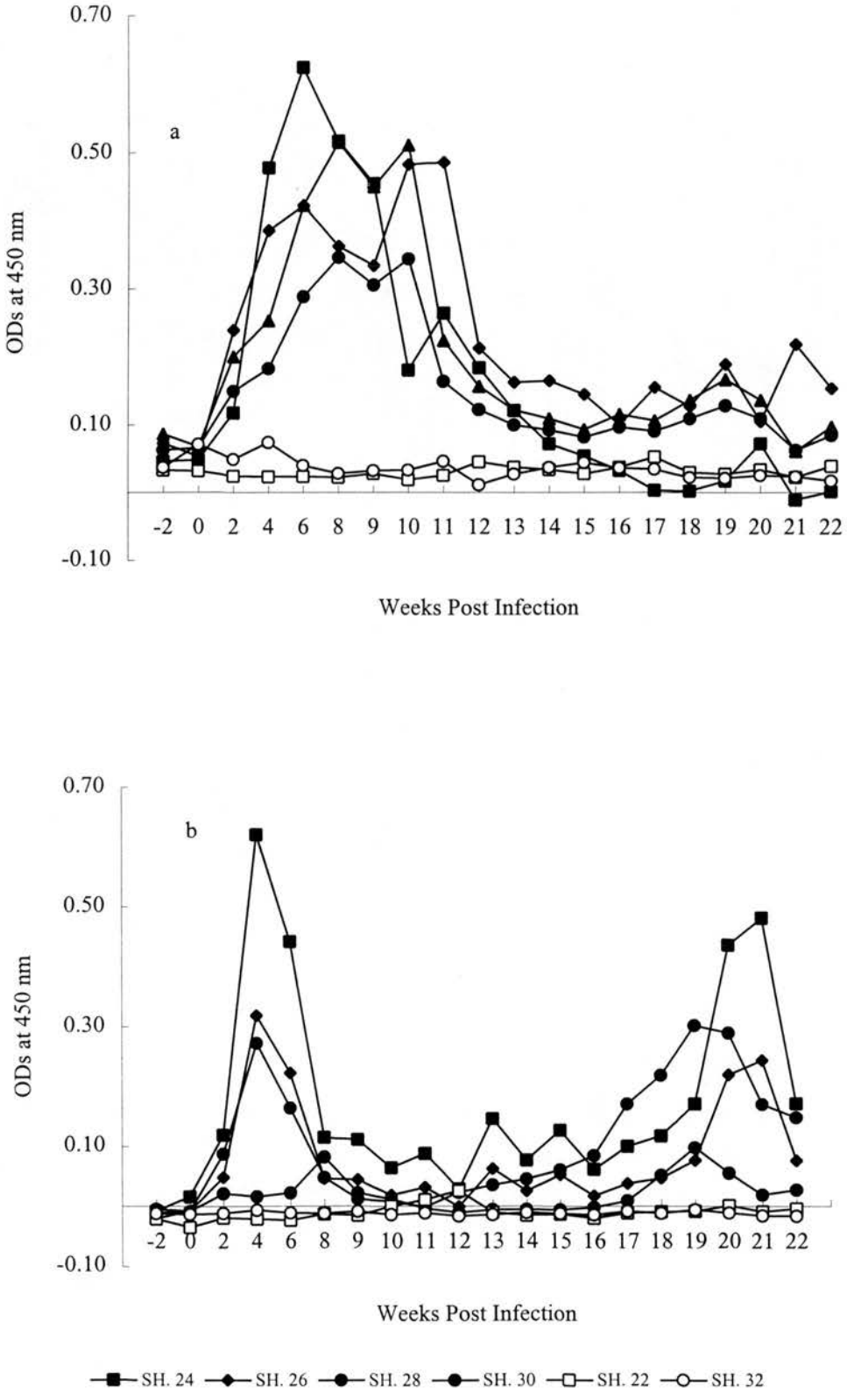


Figure 4.50: The adjusted ELISA OD (450 nm) values of IgG_2 (a) and IgA (b) responses of *F. hepatica* infected sheep (24, 26, 28 and 30) and uninfectedcontrol sheep (22 and 32) to FhESP

4.2.4 Experiment 3: *F. gigantica* (Kenyan strain) Infection in Sheep

The antibody response to Fg-E/S was easily detected in the sera of *F. gigantica* infected animals for total Ig and IgG_1, but IgM, IgG_2 and IgA values were lower.

The infected sheep showed an increase in total Ig levels from 2 wpi. with a peak at 13 wpi. The mean OD levels remained high throughout the 22 weeks of infection (Figure 4.51a). The IgG_1 isotype responses to Fg-E/S were strongest in the infected sheep after 2 wpi. with peak at 10 wpi. Although the infected sheep response started to decease by 13 wpi. the OD values remained higher than uninfected sheep until the end of experiment (Figure 4.51b).

The IgM response to Fg-E/S in infected sheep was first detected at 2 wpi. remaining elevated until the end of experiment (Figure 4.51c).

Slight IgG_2 responses to Fg-E/S were first detected at 2 wpi. remained above the uninfected up to the end of experiment 22 wpi. (Figure 4.52a).T-test analysis showed that the *F. gigantica* infected sheep IgG_2 response to Fh-E/S was significantly higher than the non infected sheep by 4 wpi.

IgA response to Fg-E/S was detected by 4 wpi. but was back to the levels of the uninfected sheep by 8 wpi. to the end of infection. The mean OD values *F. gigantica* infected animal appear to be above that of uninfected sub-group only weeks 4 to 6 of infection. The rest of the weeks analysed seem to indicate no difference between the uninfected and the infected sheep (Figure 4.52b). The data is shown in appendix tables 4.62-4.66

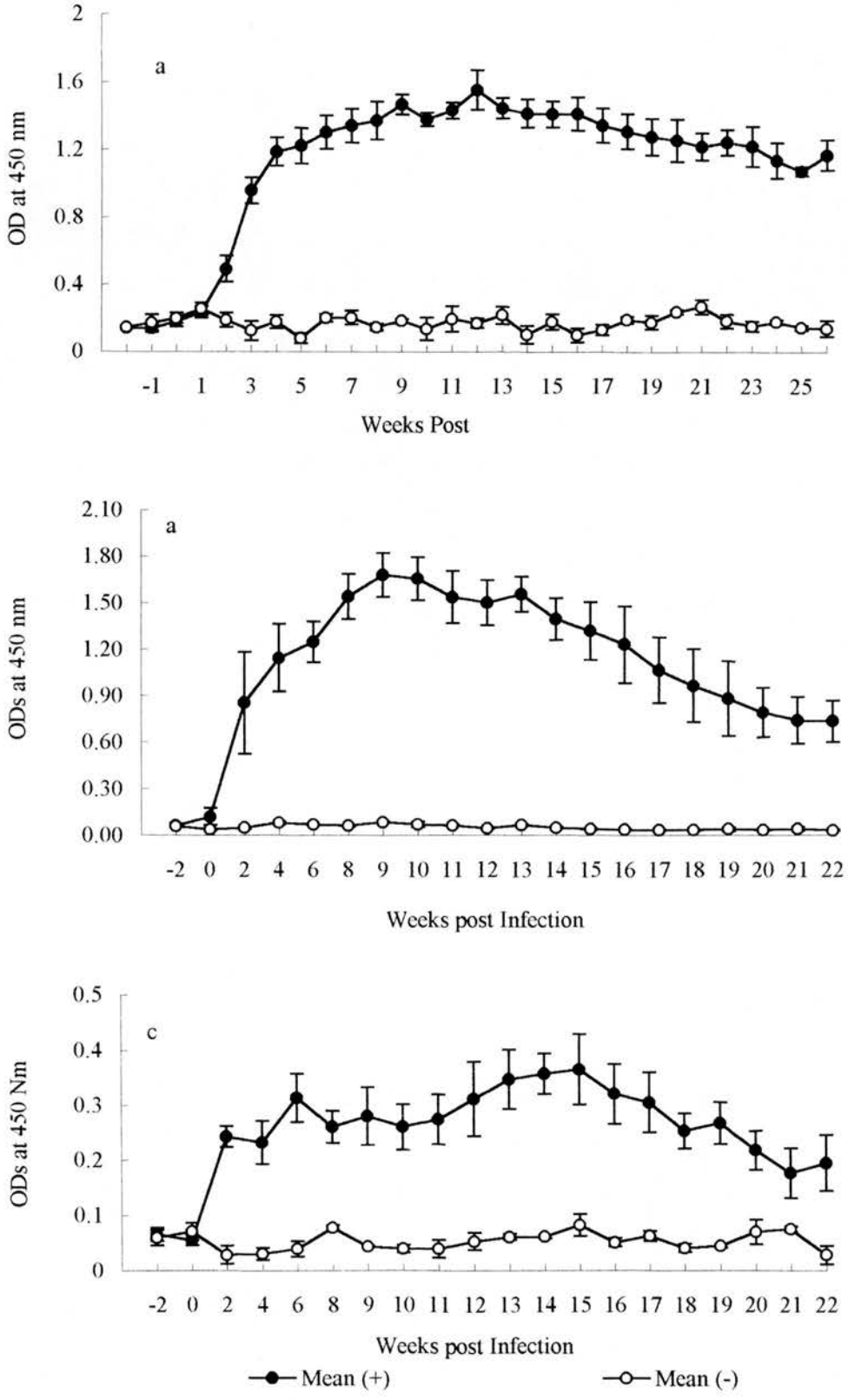


Figure 4.51: The adjusted mean ± SEM ELISA OD (450 nm) values of total Ig (a), IgG_1 (b) and IgM (c) responses of *F. gigantica* infected sheep (Mean (+)) and uninfected control sheep (Mean (-) to FgESP

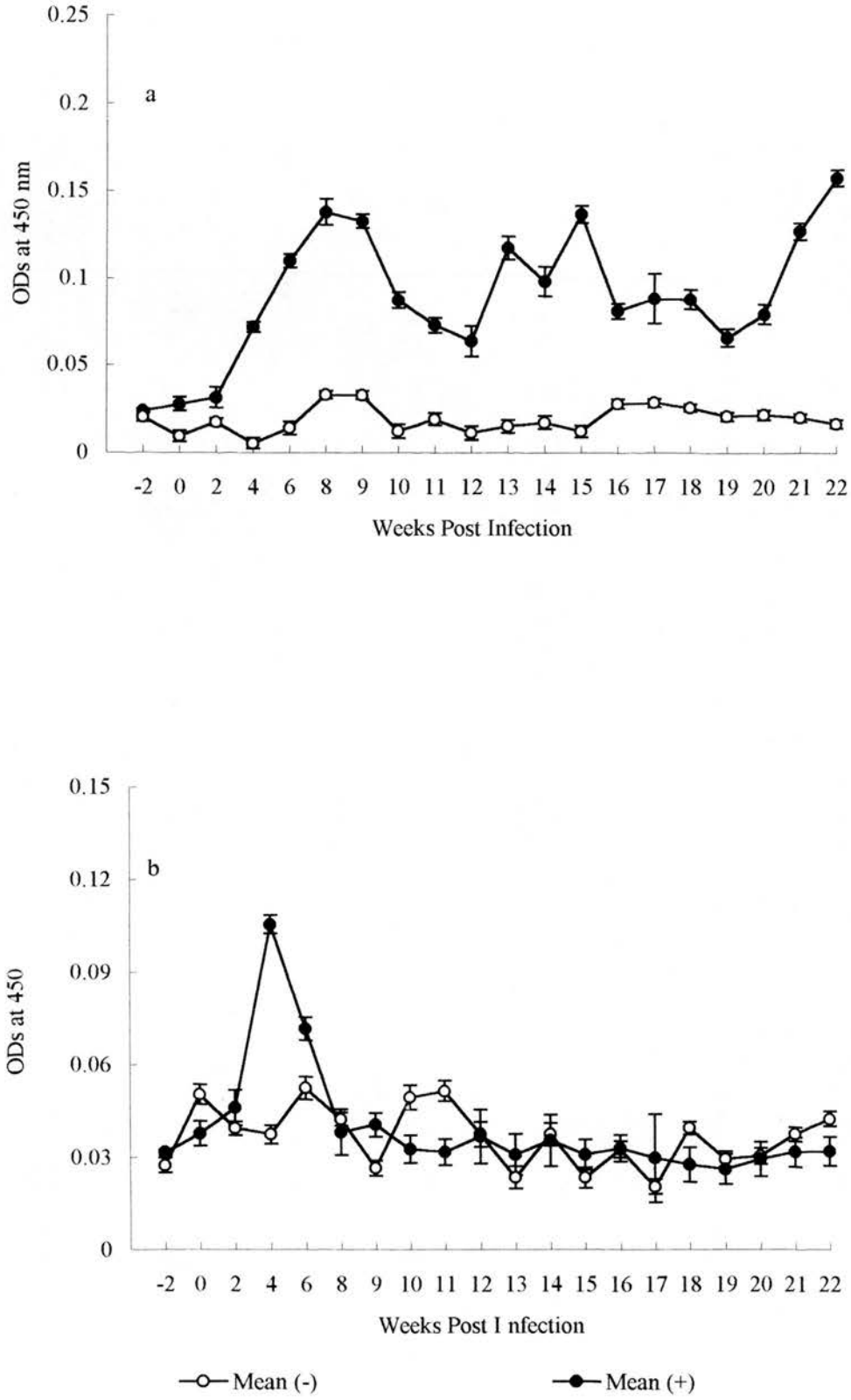


Figure 4.52: The adjusted Mean ± SEM ELISA OD (450 nm) values of IgG_2 (a) and IgA (b) responses of *F. gigantica* infected sheep (Mean (+) and uninfected control sheep (Mean (-) to FgESP

4.2.5 Experiment 4: *F. gigantica* (Kenyan strain) Infection in Sheep

The antibody response to Fg-E/S was easily detected in the sera of *F. gigantica* infected sheep for total IgG, IgG1 IgM and IgG_2 but IgA very poorly detected.

The infected sheep showed an increase in total Ig levels first at 3 wpi. for sheep 23 and 29 and 4 wpi. for sheep 25 and 27. The response reached the peak at 9 wpi. and remained high up to culling for sheep 23 and 25 and up to the end of experiment for sheep 27 and 29. in 13 wpi. (Figure 4.53a).

The mean IgG_1 isotype responses to Fg-E/S were detected in all the infected sheep from +4 wpi. peaking at +6 wpi. (Figure 4.53b) and declined +10-12 wpi. continued to reduce to near background levels by the end of the experiment.

The IgM response to Fg-E/S in infected sheep (Figure 4.53c) started 2 wpi. for sheep 27 and 29 and 4 wpi. for the rest of infected sheep reducing to the background levels by the end of experiment.

Clear IgG_2 response to Fg-E/S starting from 6 wpi. The initial response fell sharply and there was again a increase in response in all infected sheep 15 wpi. remaining high up the end of experiment (Figure 4.54a).

IgA response to Fg-E/S was relatively poor displaying a biphasic response in only two sheep (23 and 29), this was not related to either infective dose or fluke recovery (Figure 4.54b).

The adjusted mean total Ig ELISA assay results and the isotype-specific antibody responses to Fg-E/S of *F. gigantica* infected sheep 23, 25, 27, and 29 and uninfected control sheep 21 and 31 are presented in Appendix table 4.67-4.69.

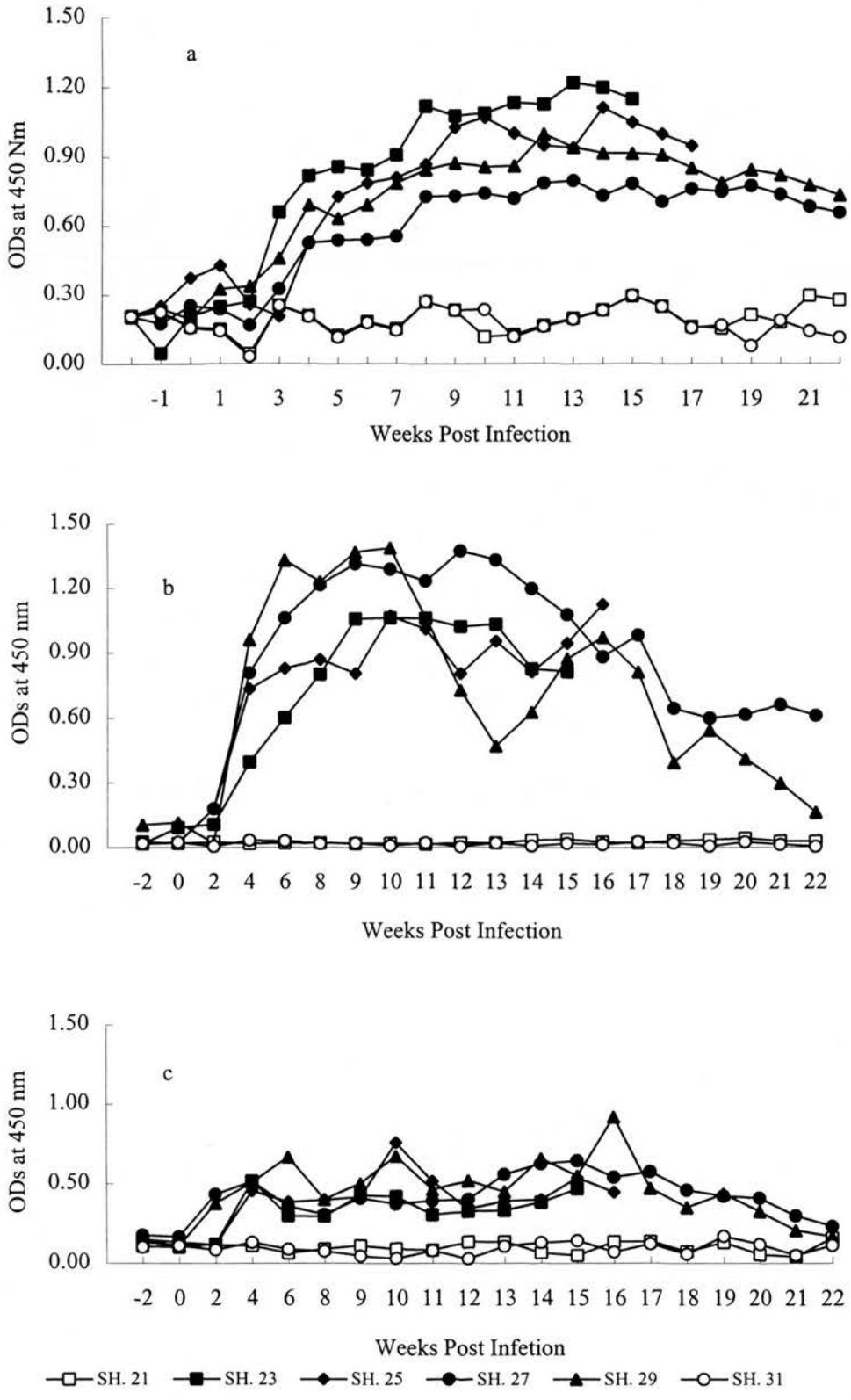


Figure 4.53: The adjusted ELISA OD (450 nm) values of Total IgG (a), IgG1 (b) and IgM (c) responses of *F. gigantica* infected sheep (23, 25, 27 and 29) and uninfected control sheep (21 and 31) to FgESP

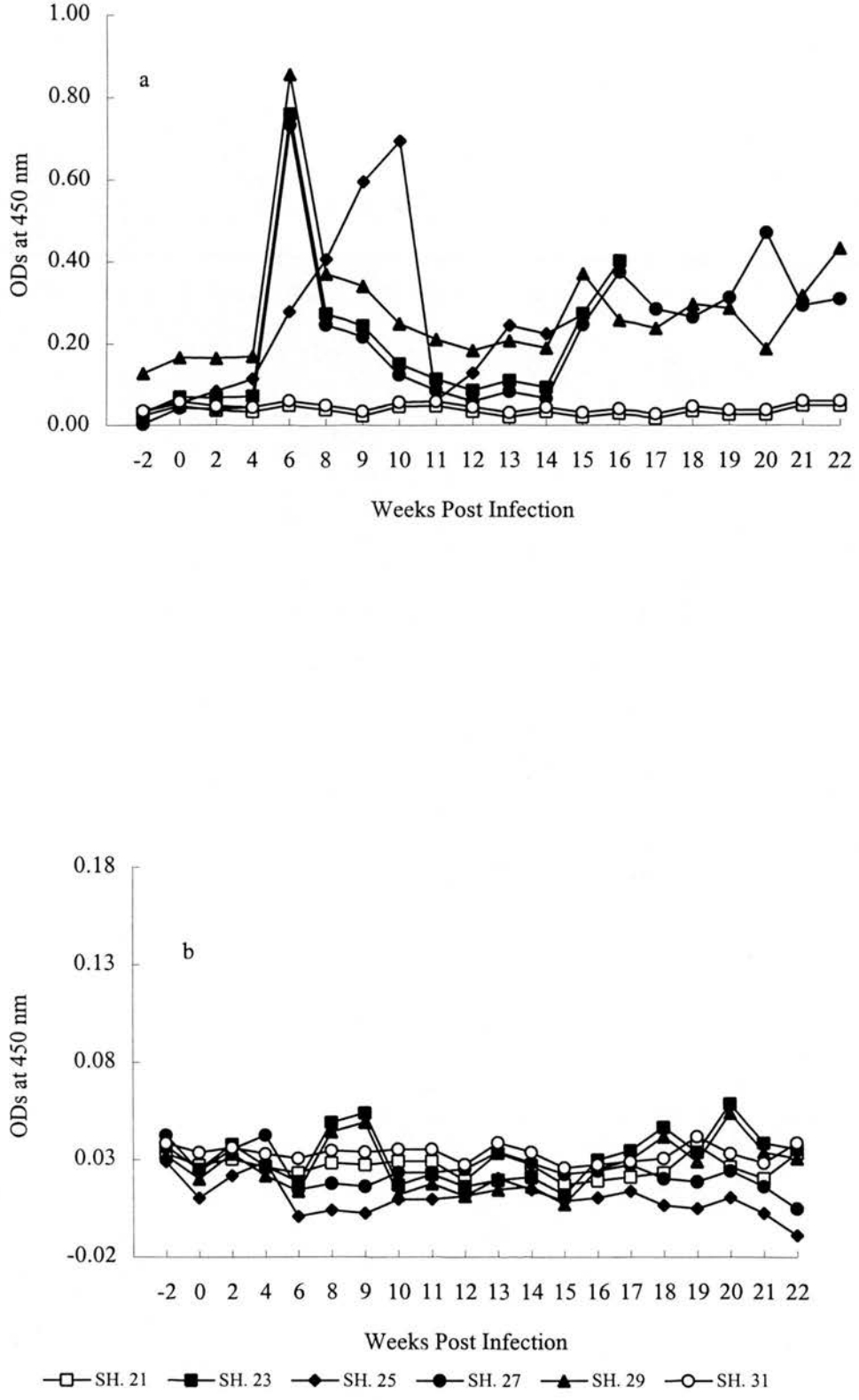


Figure 4.54: The adjusted ELISA OD (450 nm) values of IgG2 (a) and IgA (b) responses of *F. hepatica* infected (23, 25, 27 and 29) sheep and uninfected control sheep (21 and 31) to FgESP

4.2.6 Experiment 5: *F. hepatica* (Peru strain) infection in cattle

These cattle were either given primary infection as indicated or an infection and a challenge (Calf 15c and 23c) (see Table 4.5 in section 4.1.5)

All the infected calves showed an increase in total Ig levels from +4 wpi., remaining high thereafter.

IgG_1 isotype responses to Fh-E/S were stronger in all the infected calves as with total Ig, the IgG_1 response was detected 3-4 wpi. and remained unchanged throughout the experimental period (Figure 4.55a-b). In calf 15c the circulating IgG_1 antibody rose markedly after challenge infection but there was little change in calf 23c (Figure 4.55a)

IgM responses were detected +2 wpi. peaking +3-5 wpi. and remained high throughout the experiment (Figure 4.55c).

IgG_2 levels rose slowly from +9 wpi. increasing gradually till the end of the experiment. There was no marked change after challenge infection in calves 15c but calves 23c may have shown a slight rise(Figure 4.56a).

A sharp increase in IgA response to Fh-E/S was noticed in calf 23c 5 wpi. after the primary infection and 6 weeks after challenge infection. Calf 15c showed an increased response after a challenge infection while unchallenged calf 14c only responded 31 wpi. The adjusted data in presented in Appendix tables 4.70-4.74.

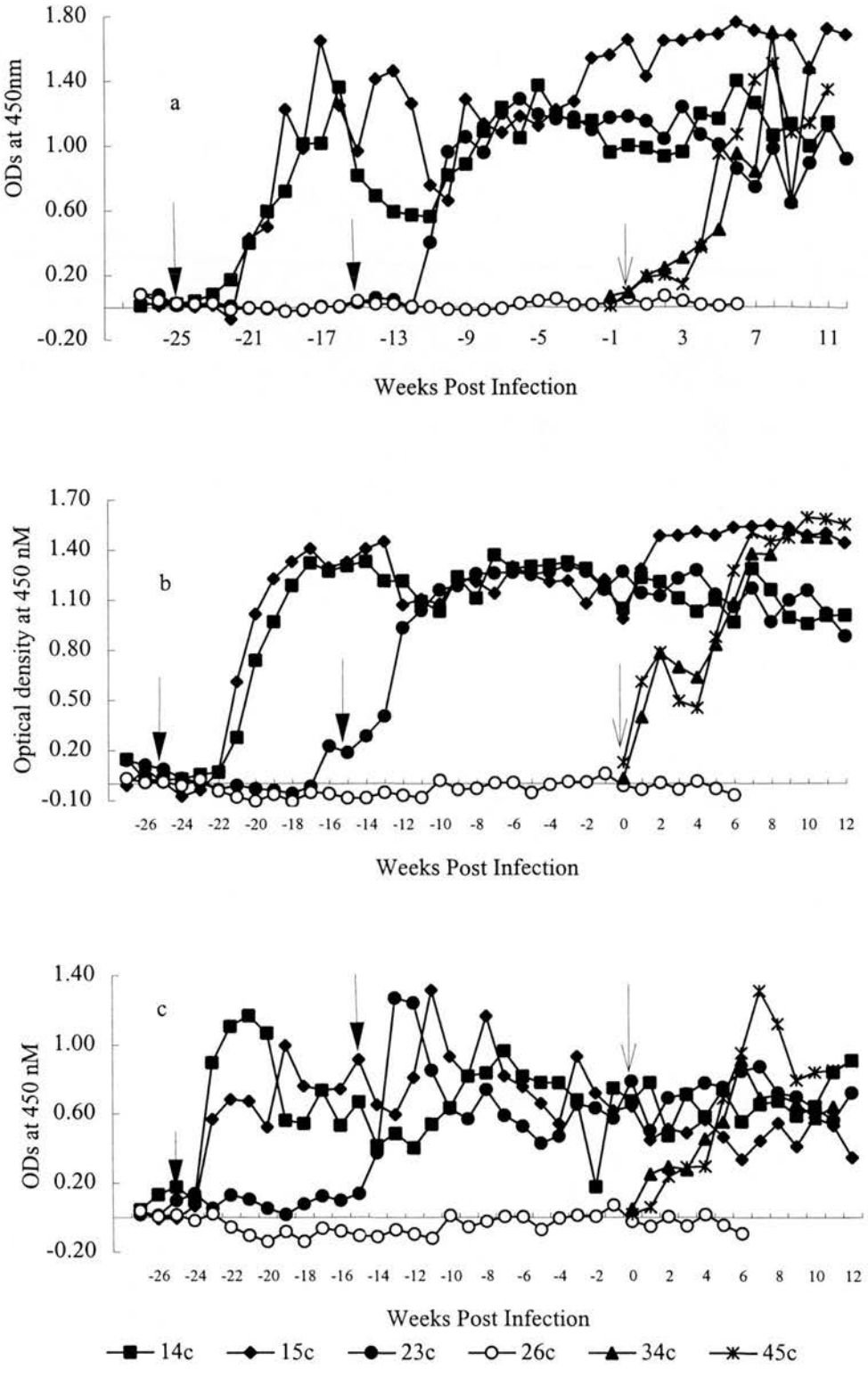


Figure 4.55: The adjusted ELISA OD (450 nm) for total Ig (a), IgG1 (b) and IgM (c) responses of *F. hepatica* infected calves (14, 15, 23, 34 and 45) and uninfected control calf (26) calves to FhESP

Primary infection Challenge infection

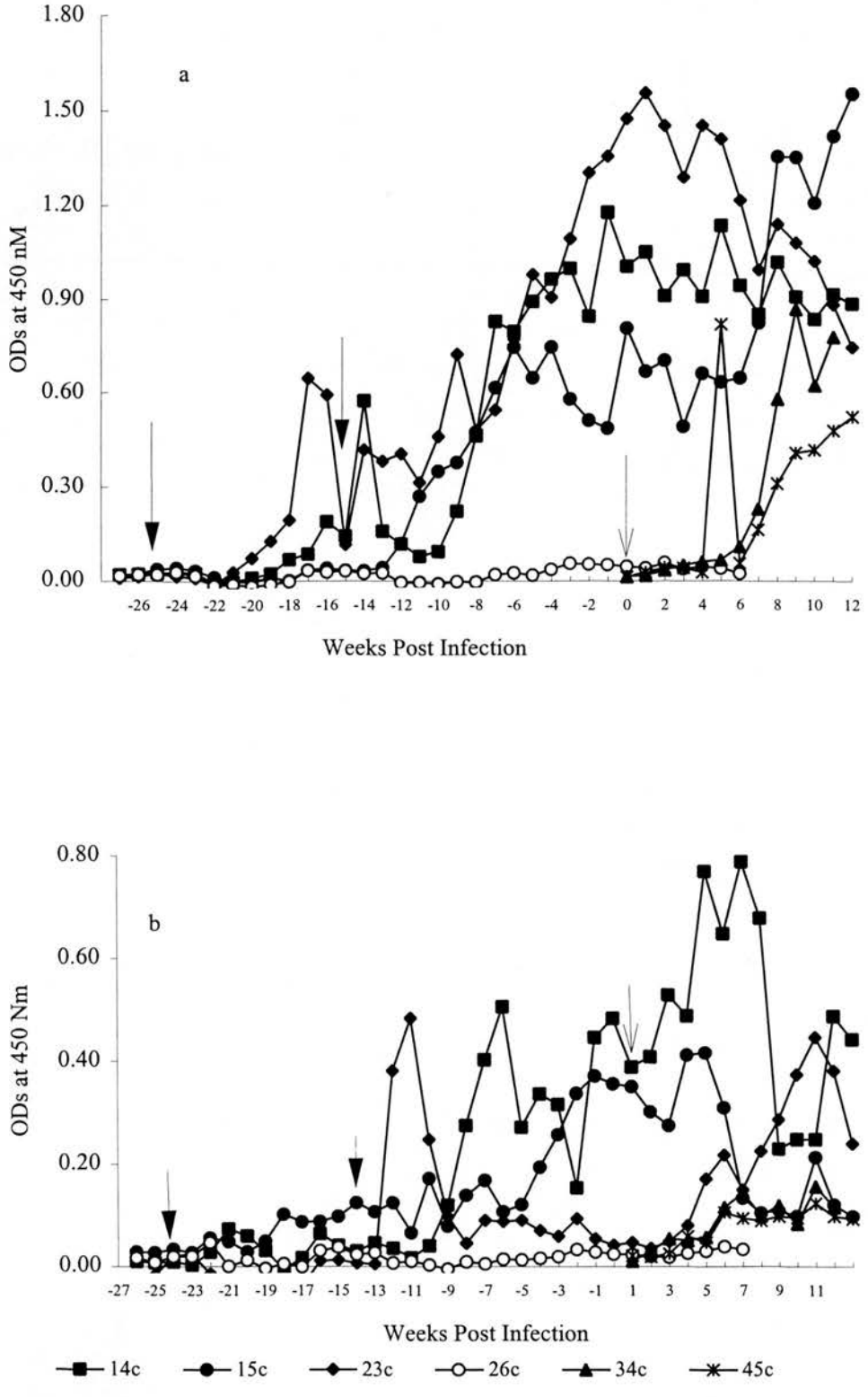


Figure 4.56: The adjusted ELISA OD (450 nm) values of IgG2 (a) and IgA (b) responses of *F. hepatica* infected calves (14c, 15c, 23c, 34c and 45c) and uninfected control (calf 26c) to FhESP

Primary infection Challenge infection

4.2.7 Experiment 6: *F. gigantica* (Kenya strain) Infection in Cattle

Following infection, the Fh-E/S antibodies were easily detected in the sera of *F. gigantica* infected calves for both total Ig and the four subclasses, IgG1, IgM, IgG_2 and IgA in varying degrees and time of infection (Figure 4.57-4.58).

All the infected calves showed an increase in total Ig levels from +3 wpi. peaking +5-7 wpi. and remained high throughout the experiment (Figure 4.57a).

Serum IgG_1 responses rose sharply 3-4 wpi. and remained high up to 27 wpi. apart from calf 24 whose response drooped to the level of the control sheep (Figure 4.58b).

IgM response to Fg-E/S rose by +2-3 wpi. and reached the peak in 5 wpi. Calves 22 and 23 showed very strong fluctuation and declined to almost the levels of the uninfected calf 26 by 26 wpi. Calf 24 on the other hand showed less fluctuation and OD's remained high throughout the weeks experiment (Figure 4.57c).

Circulating IgG2 antibody response to Fg-E/S in calves 22 and 23 was noticed from 5 wpi. but appeared very strong in calf 24 with a gradual increase until the end of monitoring period (Figure 4.58a).

IgA response to Fg-E/S in infected calves appeared suddenly late at 22 wpi. it fluctuated but with a clear increasing trend until the end of experiment (Figure 4.58b). The adjusted data in presents in Appendix tables 4.70-4.74.

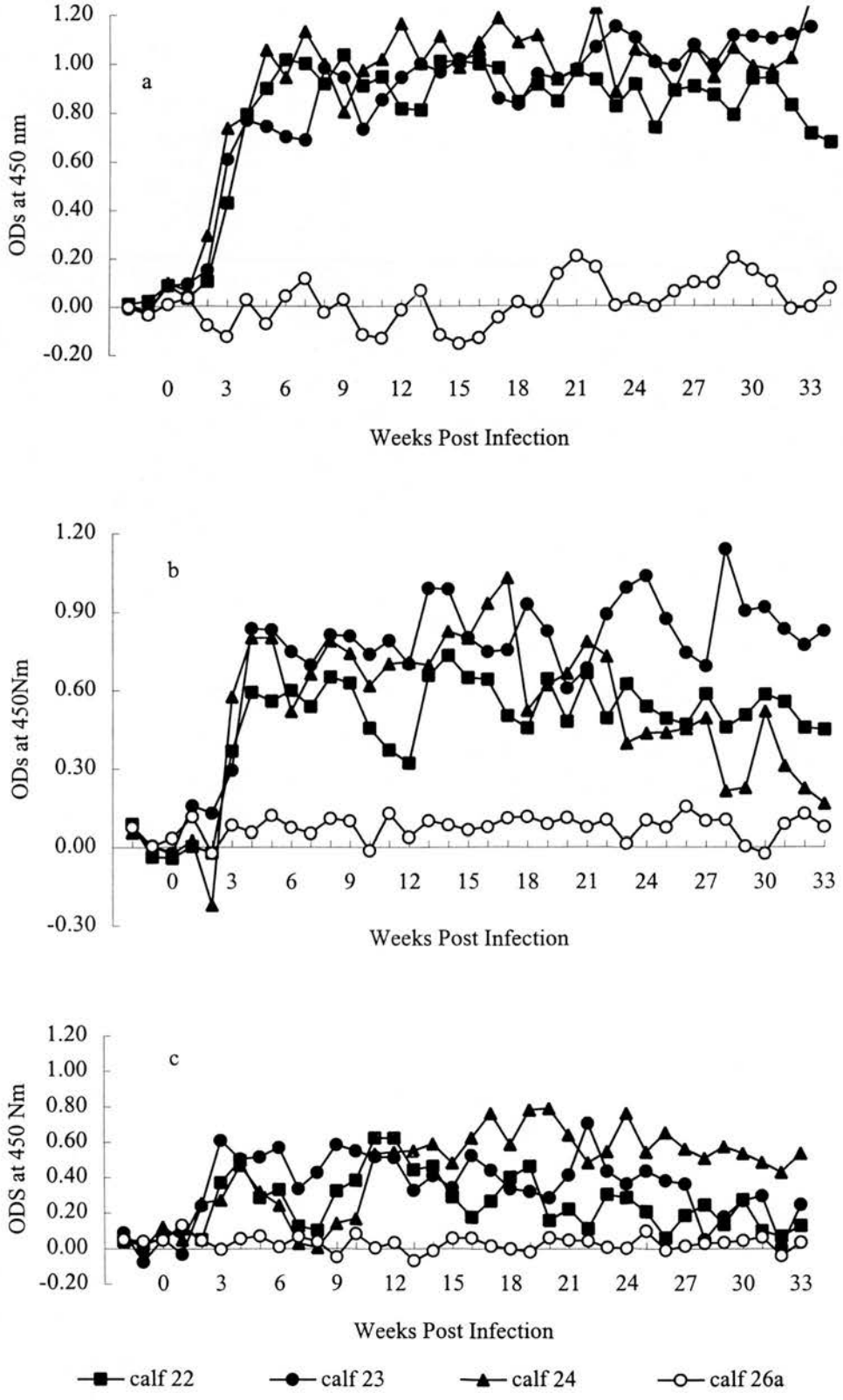


Figure 4.57: The adjusted ELISA OD (450 nm) for total Ig (a), IgG1 (b) and IgM (c) responses of *F. gigantica* infected calves (22,23 and 24) and uninfected control (calf 26) to FgESP

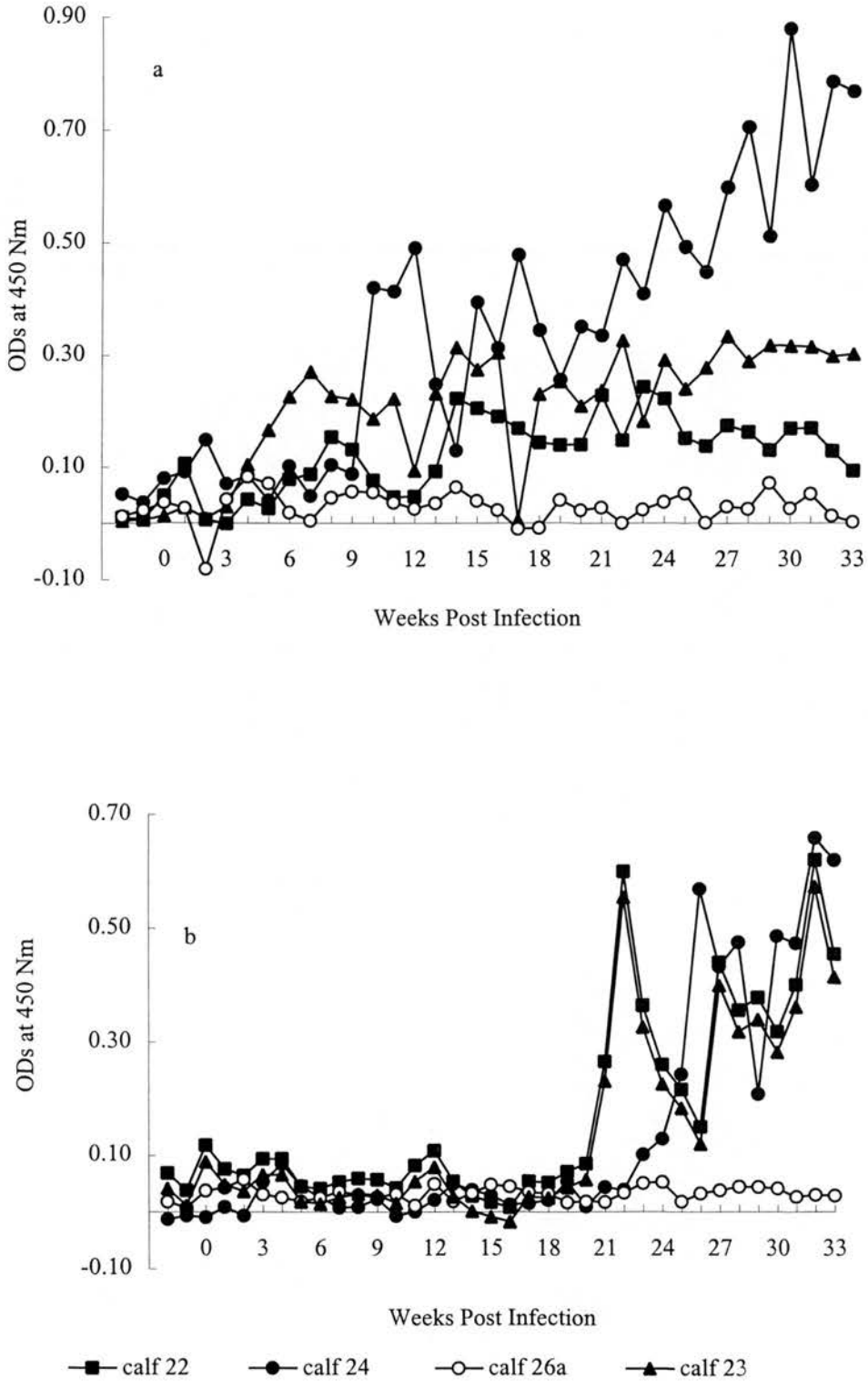


Figure 4.58: The adjusted ELISA OD (450 nm) values for IgG2 (a) and IgA (b) responses of *F. gigantica* infected calves (22, 23 and 24) and uninfected control (calf26) to FgESP

4.3 SERUM ANTIBODY RESPONSES AGAINST *F. HEPATICA* CATHEPSIN-L1 CYSTEINE PROTEASE (FH-CATHEPSIN)

4.3.1 Determination of Optimum Assay Conditions by Titration.

The results for titrations to determine optimum antigen serum, the monoclonal antibody and the conjugate dilution are summarised in Tables 4. 11 - 4.13. The antigen concentration to be used in the ELISA assay for each of the isotypes was determined by Fh-cathepsin titration at 4, 2 and 1 μg/ml. All titrations were carried out in duplicate and the mean values calculated. The selected concentration was 1 μg/ml for all of the isotypes in either *F. hepatica* or *F. gigantica* infected sheep and cattle. Figures 4.59-4.66 are representative titrations for total Ig and IgG1 in *F. hepatica* and *F. gigantica* infected sheep and cattle for the two polyclonal (total Ig) and the monoclonal antibody (IgG_1) based detection systems. Full data is represented in Appendix tables 4.75-4.84.

Chequerboard titration for serum, monoclonal antibodies and conjugate were carried out by diluting these systems in blocking buffer using doubling serial dilution ranging from 1-4μg/ml (antigen), 1:50-1:1600 (serum), 1:20-1:320 (monoclonal antibody) and 1:1000-1:32,000 (conjugate). The chosen antigen concentration, serum, monoclonal antibody and conjugate dilution were used in all subsequent sequential screenings. Dilutions were selected on the basis of optimising the signal to background ratio. The two positive sera P1 and P2 were taken from *F. hepatica* or *F. gigantica* infected animals and corresponded to 8, 9 or 10 wpi (P1) and 21,22 or 23 wpi (P2) for sheep and to 7 wpi (P1) and 32 wpi (P2) for calves. These times post infection were chosen to assess responses at the middle and at the end of the experimental period and to optimise the signal to background ratio.

Table 4.11: Sheep infected with *F. hepatica* or *F. gigantica*: Optimal dilutions of serum and conjugate for polyclonal antibody system using Cathepsin-L protease from *F. hepatica* as antigen. Antigen concentration of 1µg/ml in all the sheep assays.

Assay	*F. hepatica*			*F. gigantica*		
	Fh-Cathepsin (µg/ml)	Serum	Conj.	Fh-Cathepsin (µg/ml)	Serum	Conj.
Total Ig	1	1:400	1:4000	1	1:200	1:4000
IgM	1	1:200	1:2000	1	1:200	1:2000

Table 4.12 Sheep infected with *F. hepatica* or *F. gigantica*: Optimal dilutions of serum monoclonal antibody (McAb) and conjugate for monoclonal antibody system using Cathepsin-L protease from adult *F. hepatica* as antigen. Antigen concentration of 1µg/ml in all the sheep assays.

Assay	*F. hepatica*				*F. gigantica*			
	Fh-Cathepsin (µg/ml)	Serum	McAb	Conj.	Fh-Cathepsin (µg/ml)	Serum	McAb	Conj.
IgG_1	1	1:200	1:400	4000	1	1:200	1:400	1:4000
IgG_2	1	1:50	1:20	1000	1	1:50	1:20	1:1000
IgA	1	1:50	1:20	1000	1	1:50	1:20	1:1000

Table 4.13 Cattle infected with *F. hepatica* or *F. gigantica*: Optimal dilutions of serum and conjugate for polyclonal antibody system using Cathepsin-L protease from *F. hepatica* as antigen. Antigen concentration of 1µg/ml in all the cattle assays.

Assay	*F. hepatica*			*F. gigantica*		
	Fh-Cathepsin (µg/ml)	Serum	Conj.	Fh-Cathepsin (µg/ml)	Serum	Conj.
Total Ig	1	1:200	1:4000	1	1:200	1:4000
IgG_1	1	1:200	1:4000	1	1:200	1:4000
IgM	1	1:200	1:2000	1	1:200	1:2000
IgG_2	1	1:50	1:1000	1	1:50	1:1000
IgA	1	1:50	1:1000	1	1:50	1:1000

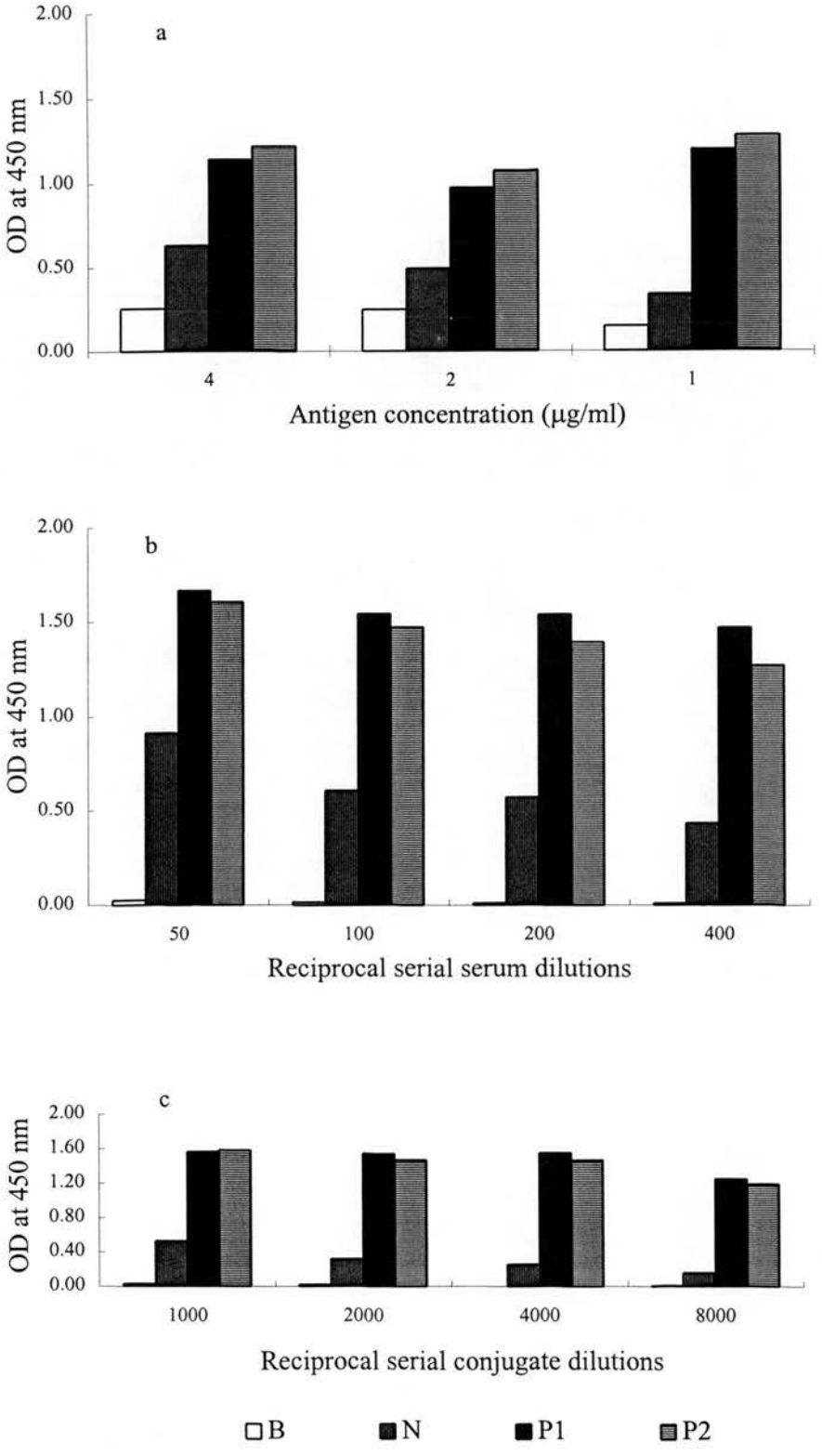


Figure 4.59: Antigen (Fh-cathepsin) (a), serum (b) and conjugate (c) titrations for total Ig for *F. hepatica* infected sheep showing the mean ELISA (450 nm) obtained negative serum (N) and two positive serum samples, positive 1 (P1) and positive 2 (P2).

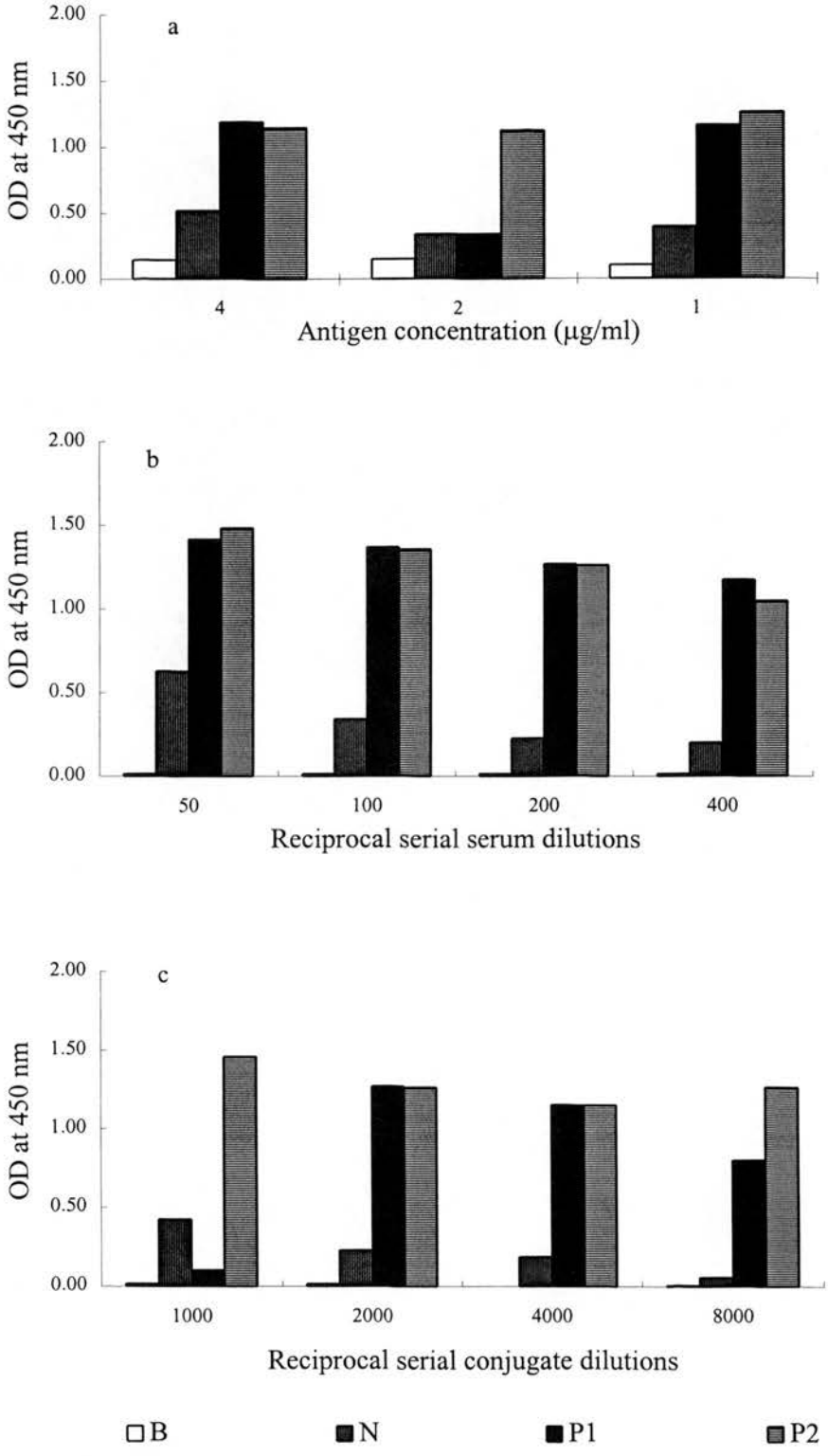


Figure 4.60: Antigen (Fh-cathepsin) (a), serum (b) and conjugate (c) titrations for total Ig for *F. gigantica* infected sheep showing the mean ELISA (450 nm) negative serum (N) and two for diluent (B), positive serum samples, positive 1 (P1) and positive 2 (P2).

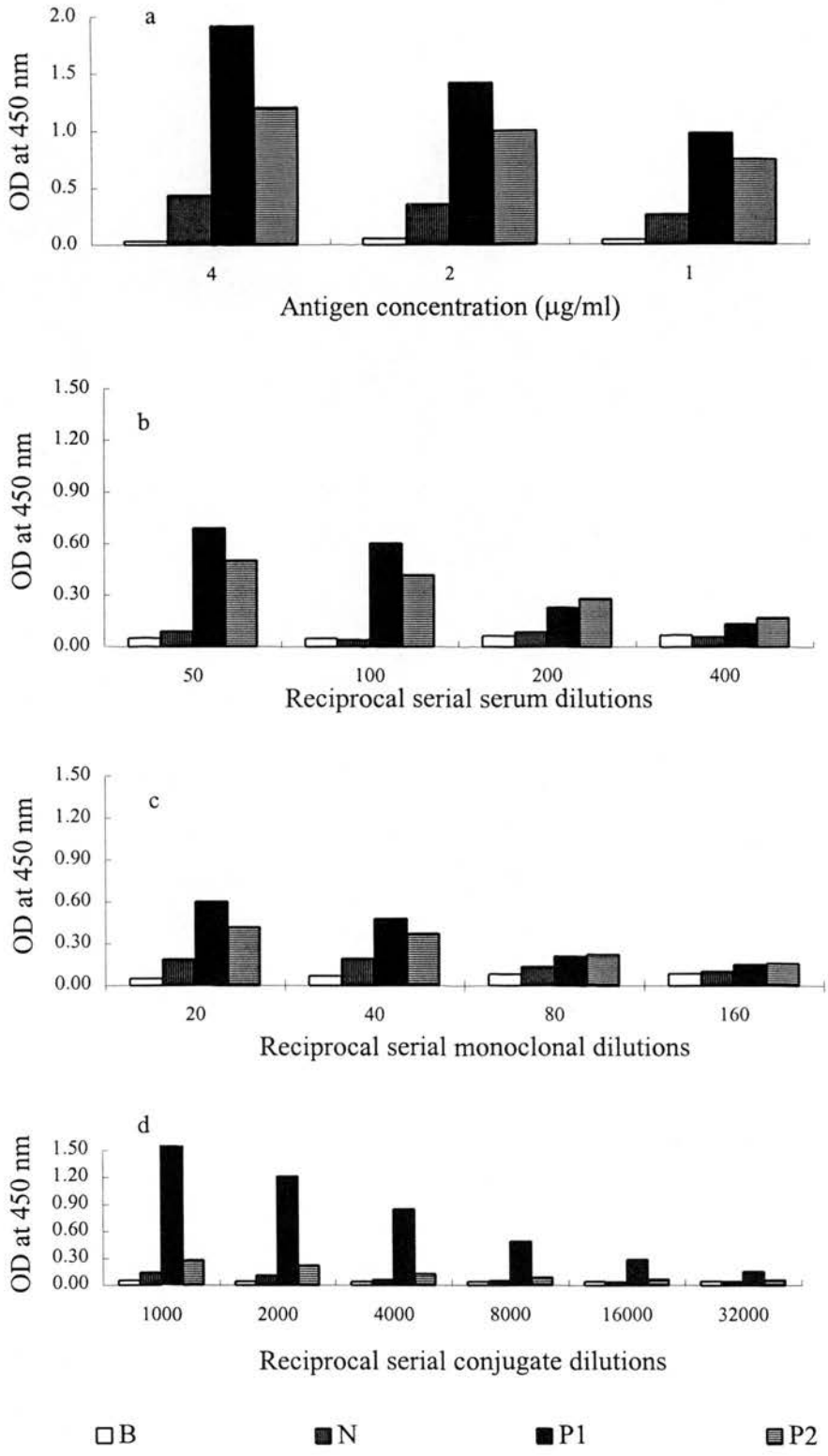


Figure 4.61 Antigen (Fh-cathepsin) (a), serum (b), monoclonal (c) and conjugate(d) titrations for IgG_1 for *F. hepatica* infected sheep showing the mean ELISA (450 nm) values obtained for diluent (B), negative serum (N) and two positive serum samples, positive 1 (P1) and positive 2 (P2).

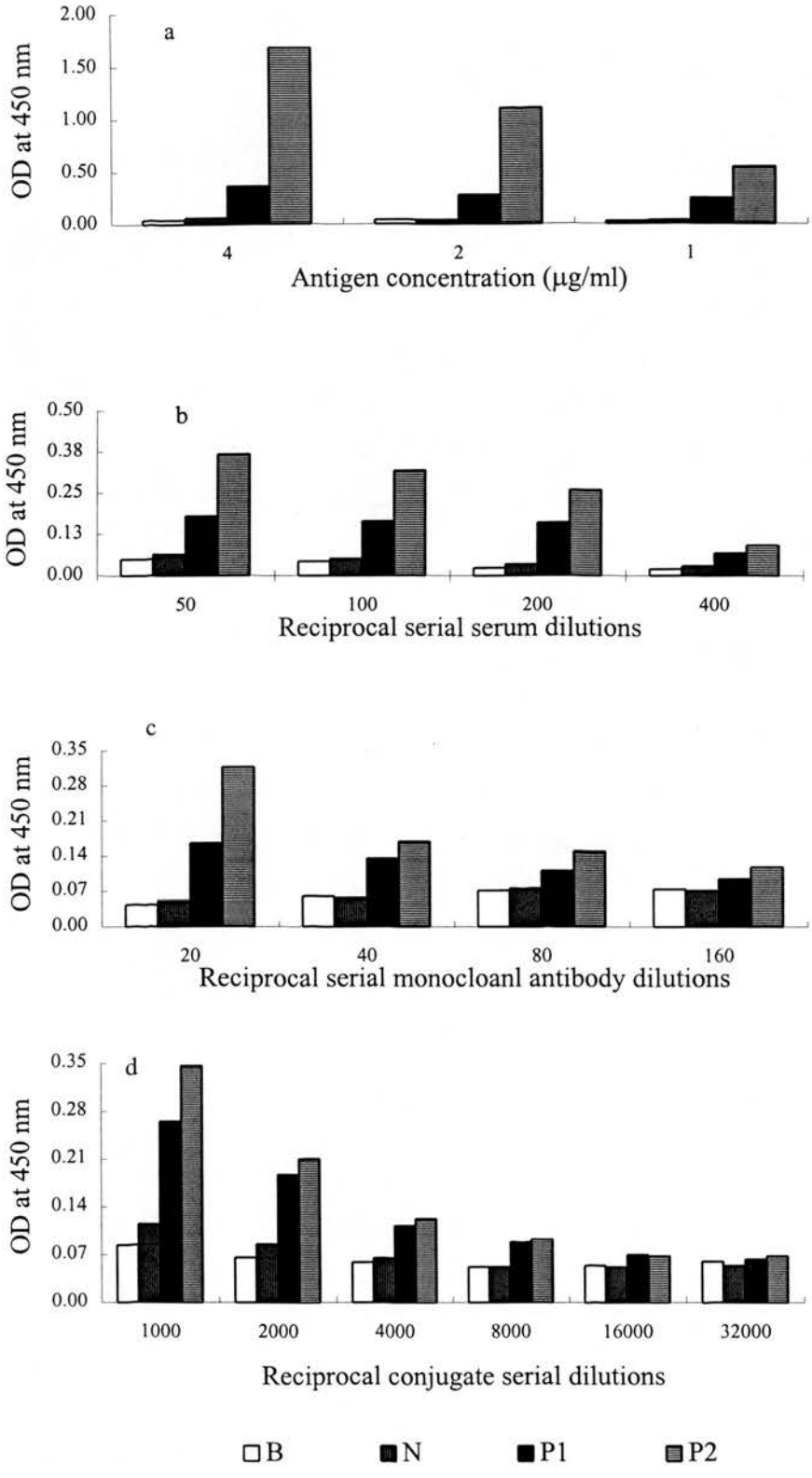


Figure 4.62: Antigen (Fh-cathepsin) (a), serum (b), monoclonal (c) and conjugate (d) titrations for IgG_1 for *F. gigantica* infected sheep showing the mean ELISA (450 nm) values obtained for diluent (B), negative serum (N) and two positive serum samples, positive 1 (P1) and positive 2 (P2).

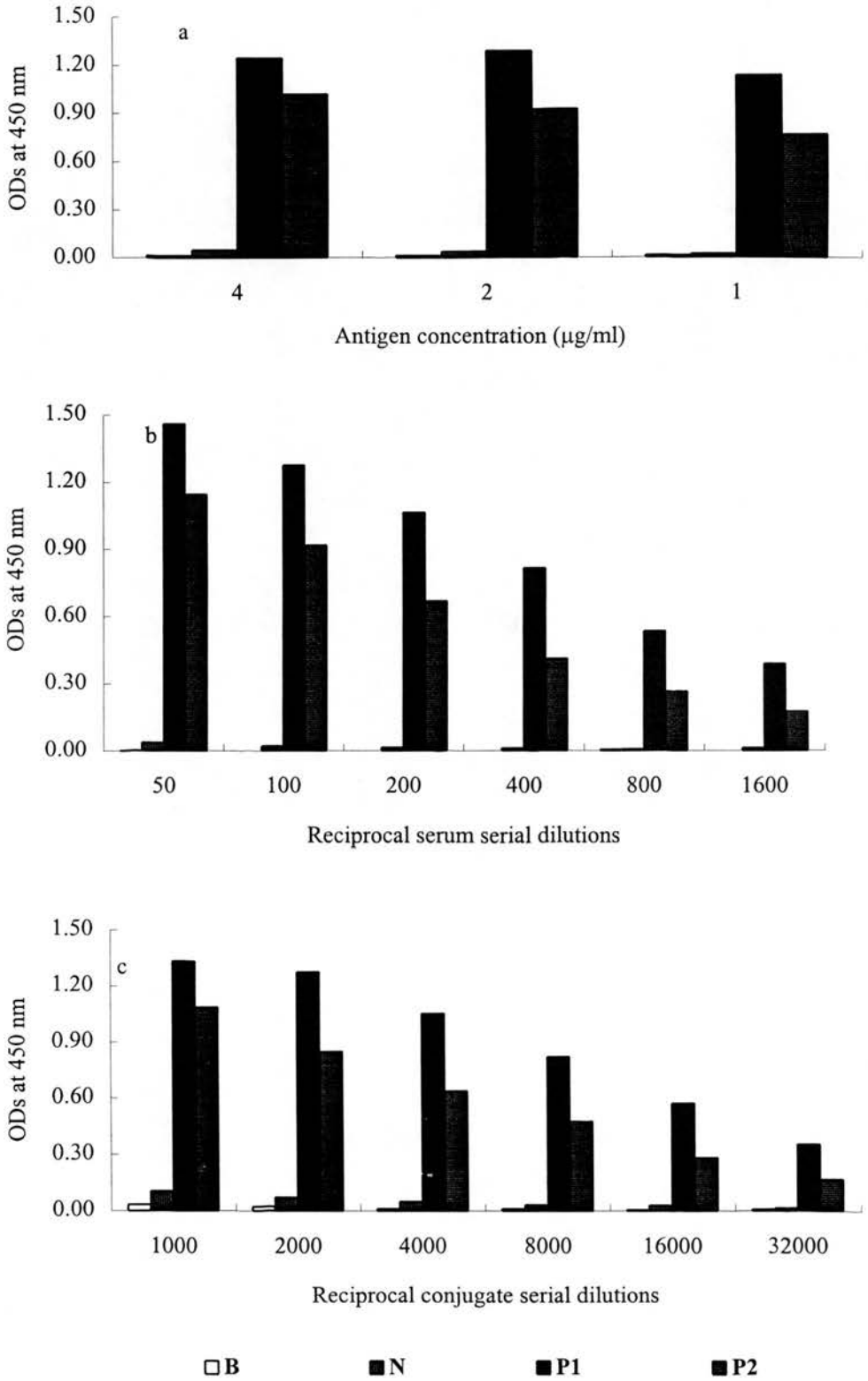


Figure 4.63: Antigen (Fh-cathepsin) (a), serum (b) and conjugate (c) titration for total Ig for *F. hepatica* infected cattle and uninfected control cattle showing the mean ELISA (450 nm) values obtained for diluent (B) negative (N) positive (P1) and positive (P2)

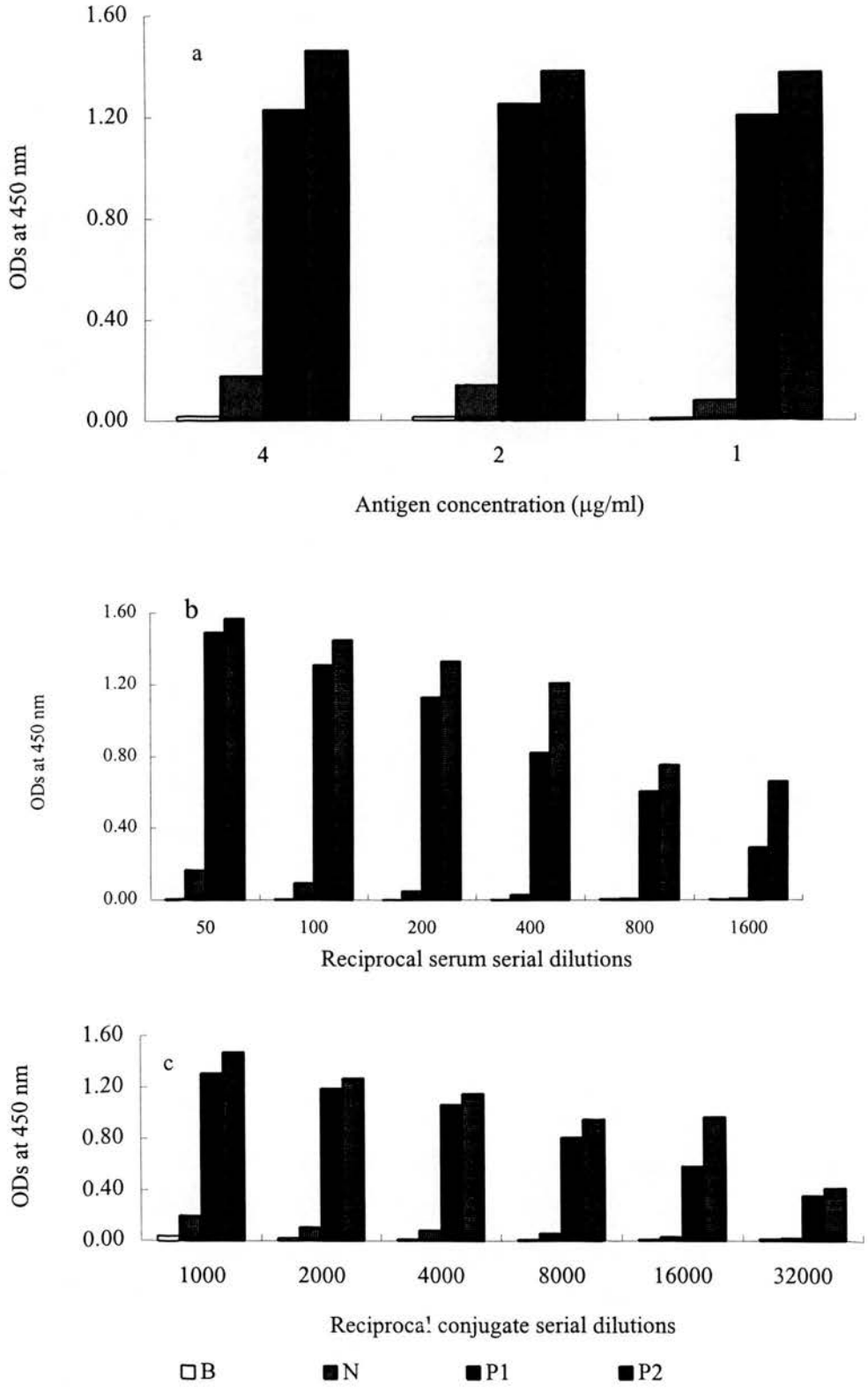


Figure 4.64: Antigen (Fh-Cathepsin) (a), serum (b) and conjugate (c) titration for total Ig for *F. gigantica* infected cattle and uninfected control cattle showing mean ELISA (450 nm) values obtained for diluent (B) negative (N) positive (P1) and positive (P2)

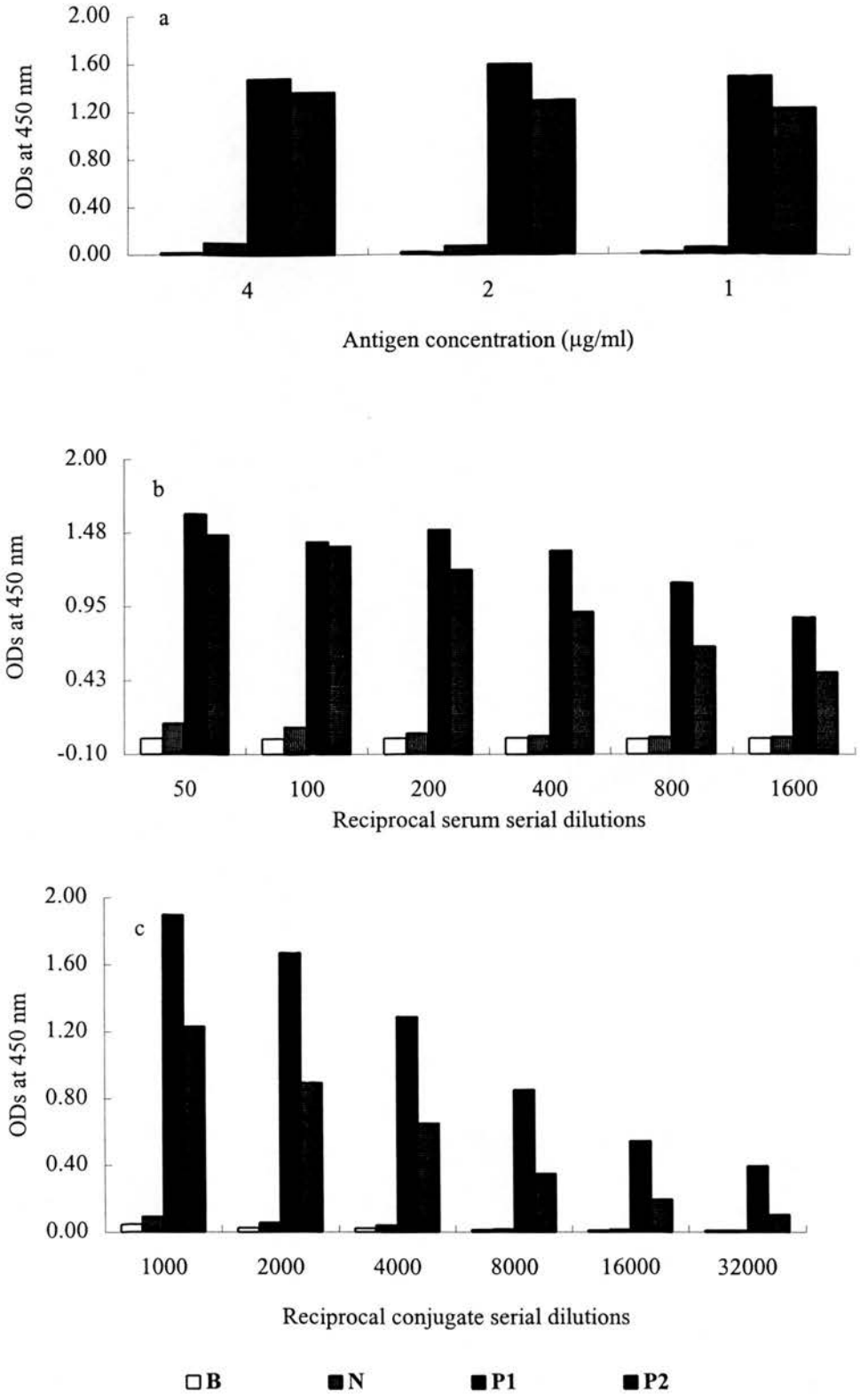


Figure 4.65: Antigen (Fh-cathepsin) (a), serum (b) and conjugate (c) titrations for IgG_1 for *F. hepatica* infected and uninfected control cattle showing the mean ELISA (450 nm) values obtained for diluent (B) negative (N) positive (P1) and positive (P2)

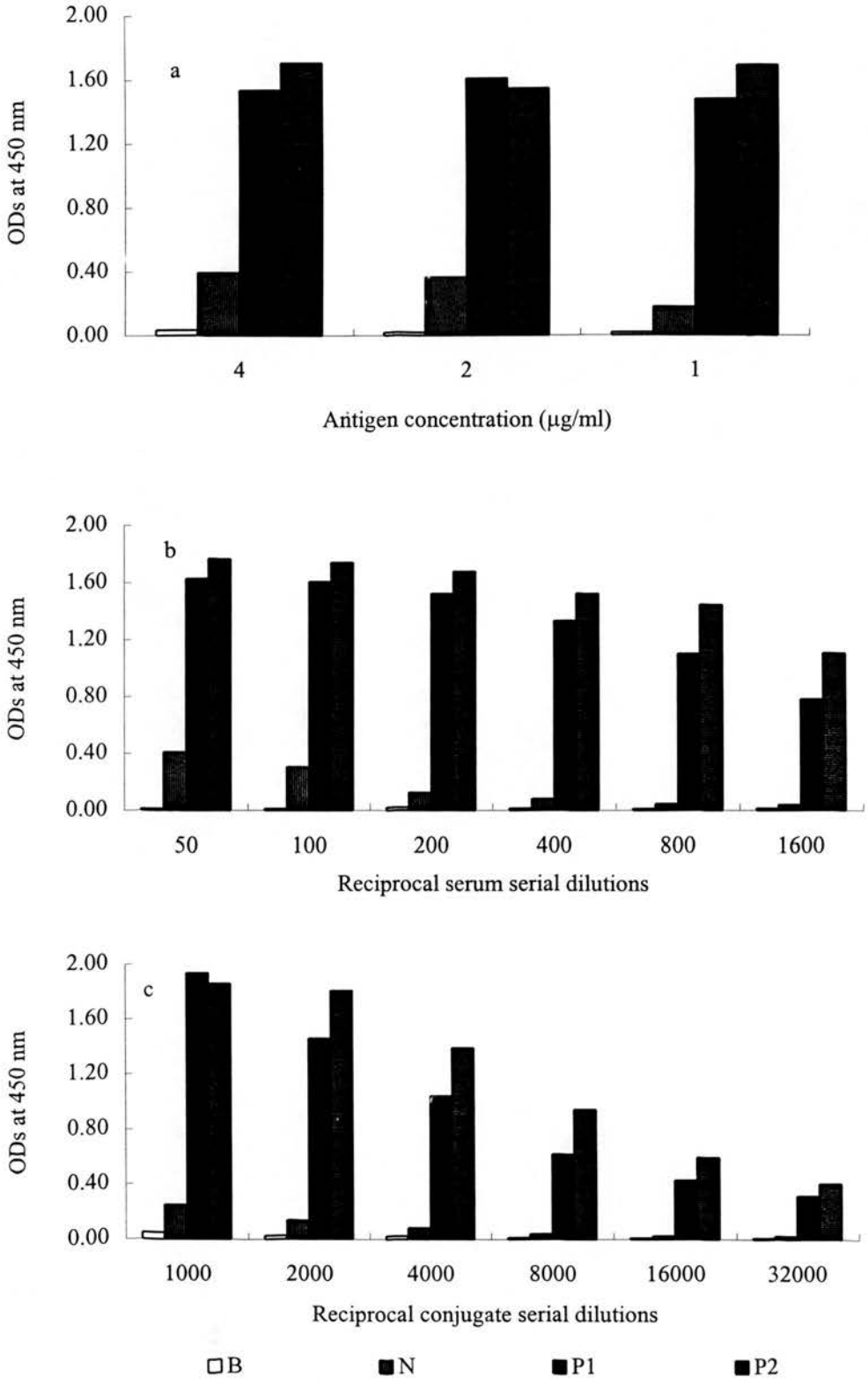


Figure 4.66: Antigen (Fh-Cathepsin) (a), serum (b) and conjugate (c) titrations for IgG_1 for *F. gigantica* infected cattle and uninfected control cattle showing the mean ELISA (450 nm) values obtained for diluent (B) negative (N) positive (P1) and positive (P2)

4.3.2 Experiment 1: *F. hepatica* (Peru And British Strain) Infection in Sheep

Antibodies response to Fh-Cathepsin were detected in the infected sheep. However there was a very poor IgG2 and IgA (Figures 4.67-4.68)

All the infected sheep showed an increase in total Ig levels from 2 wpi. with peaked OD values at week 3-7 wpi. and remained throughout experimental period, although antibody response of sheep 6 began to drop by week 12 wpi.

IgG_1 isotype responses were stronger in sheep 6 and 7 from week 2-11 wpi.

IgM levels were clearly noticeable in all infected sheep. Responses were apparent from 2 wpi. peaking 7 wpi. Sheep 6 and 7 showed strongest IgM responses.

IgG_2 responses were poor with only sheep 10 infected with 200 *F. hepatica* metacercariae showing some clear responses (Figure 4.68a).

IgA response was almost non existent but a response was noticed by 2 wpi. in sheep 5 and 6 then all the sheep 8 wpi., suggesting a biphasic response. The details of the adjusted data is in appendix tables 4.85-4.87.

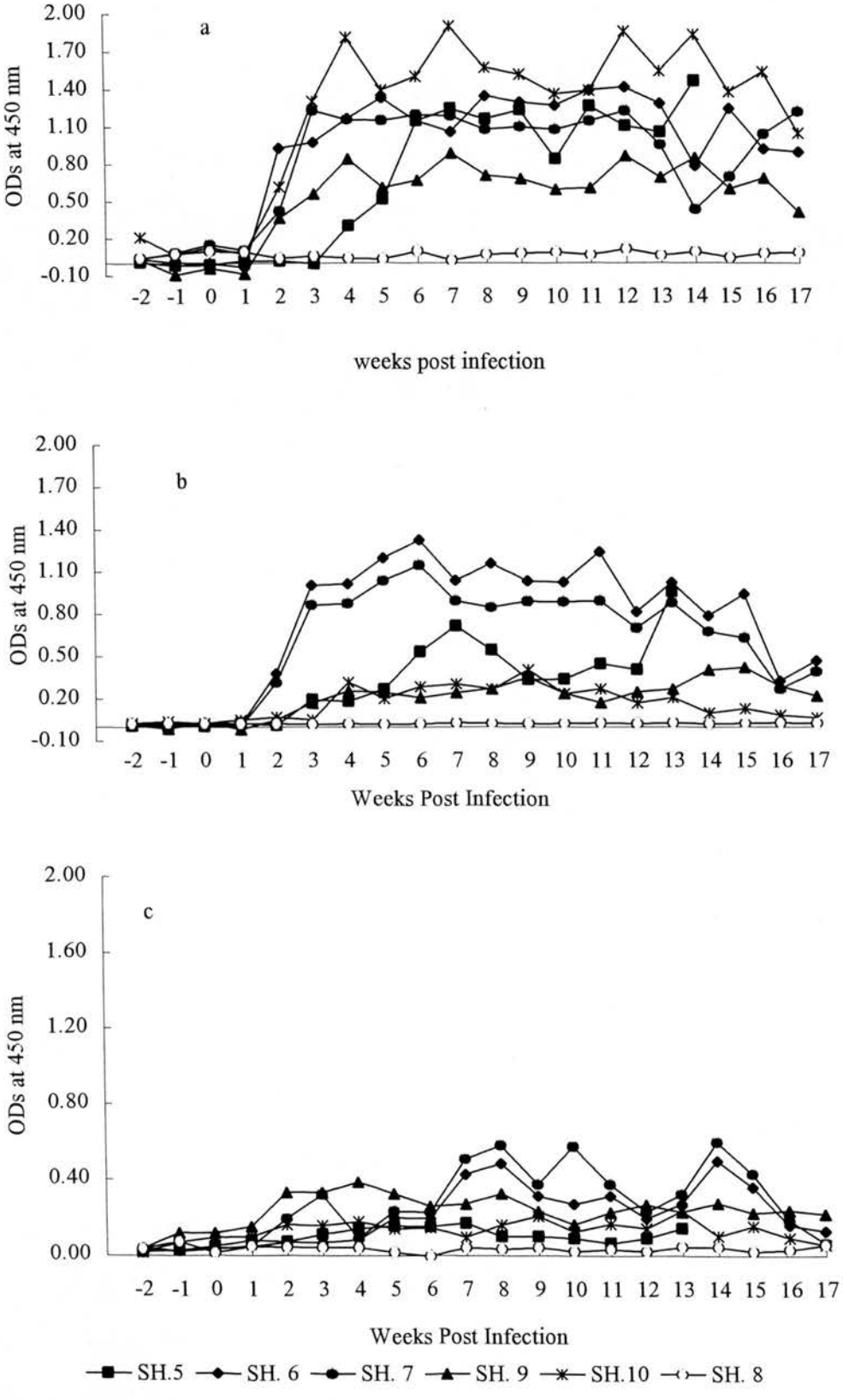


Figure 4.67: The ELISA OD (450 nm) for total Ig (a), IgG_1 (b) and IgM (c) *F. gigantica* infected sheep (5, 6, 7, 9 and 10) and uninfected sheep (8) to Fh-cathepsin

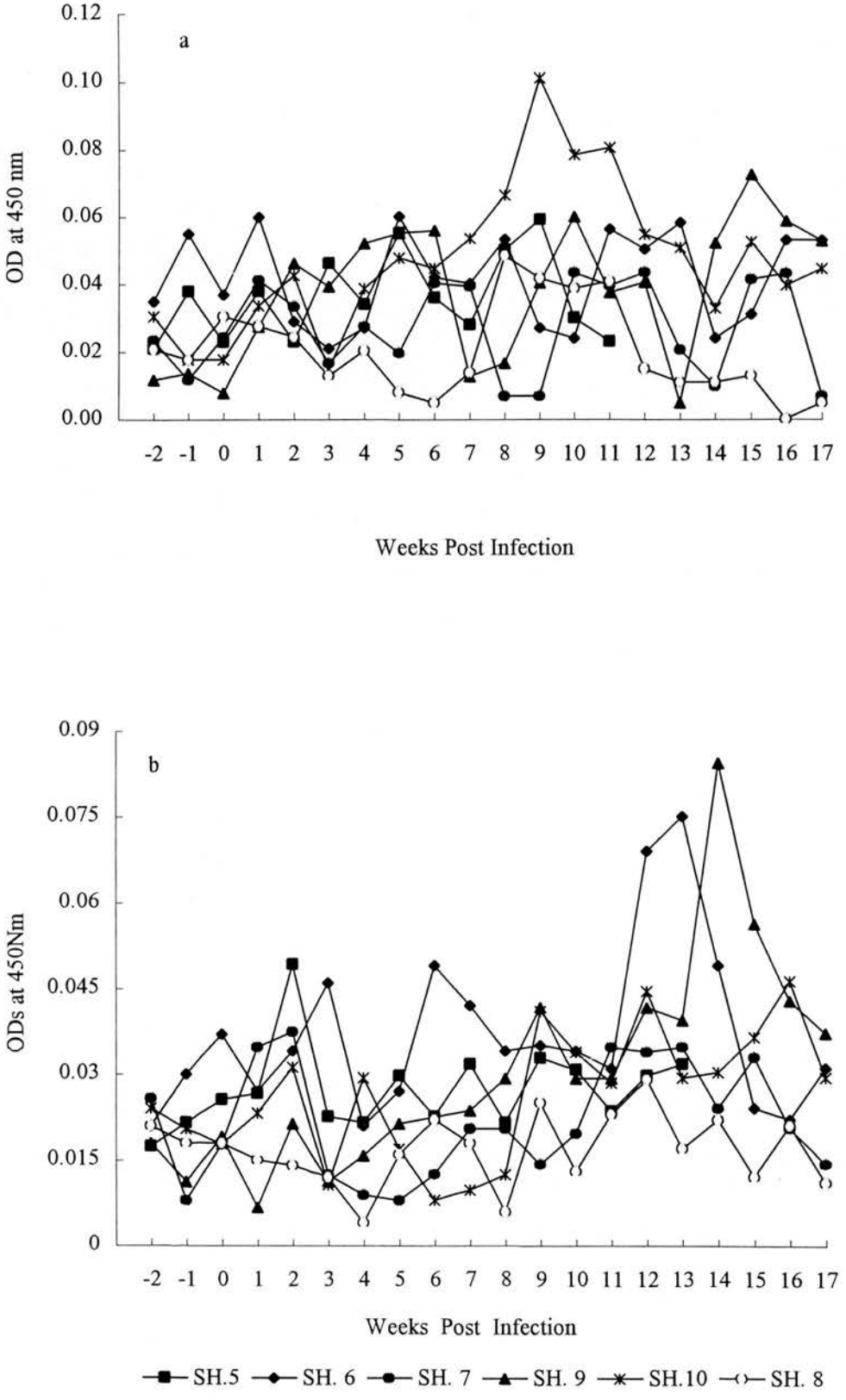


Figure 4.68: The ELISA OD (450 nm) for IgG_2 (a) and IgA (b) responses of *F. gigantica* infected sheep (5, 6, 7, 9 and 10) and uninfected control sheep (8) to Fh-cathepsin

4.3.3 Experiment 2: *F. Hepatica* (British Strain) Infection in Sheep

Antibody to Cathepsin-L protease were easily detected in *F. hepatica* infected sheep. The total Ig and IgG1 responses were most marked while IgM, IgG_2 and IgA were relatively poor (Figures 4.69-4.70).

The infected sheep showed increased total Ig levels +2 wpi. peaking at 8 wpi. remaining high throughout the experiment.

IgG_1 isotype responses to Cathepsin-L protease were first detected by +8-10 wpi. peaking at 10-20 wpi. and dropped by the end of experiment.

IgM responses was very poor although sheep 24 developed a distinct but variable IgM responses than the others showing peaks of activity at different wpi. (Figure 4.69c).

IgG_2 responses were poor in all infected sheep of this group (Figure 4.70a).. An assay was carried out to assess whether this poor response was due to the assay. It was established there was very clear response to Fh-E/S (Figure 4.50a) but not to Fh-cathepsin (Figure 4.70a).

Only a slight increase in IgA response was noted in particular in sheep 26, which displayed an early response from 2-13 wpi. (Figure 4.70b). The adjusted data to each sheep are presented in Appendix Tables 4.88-4.90.

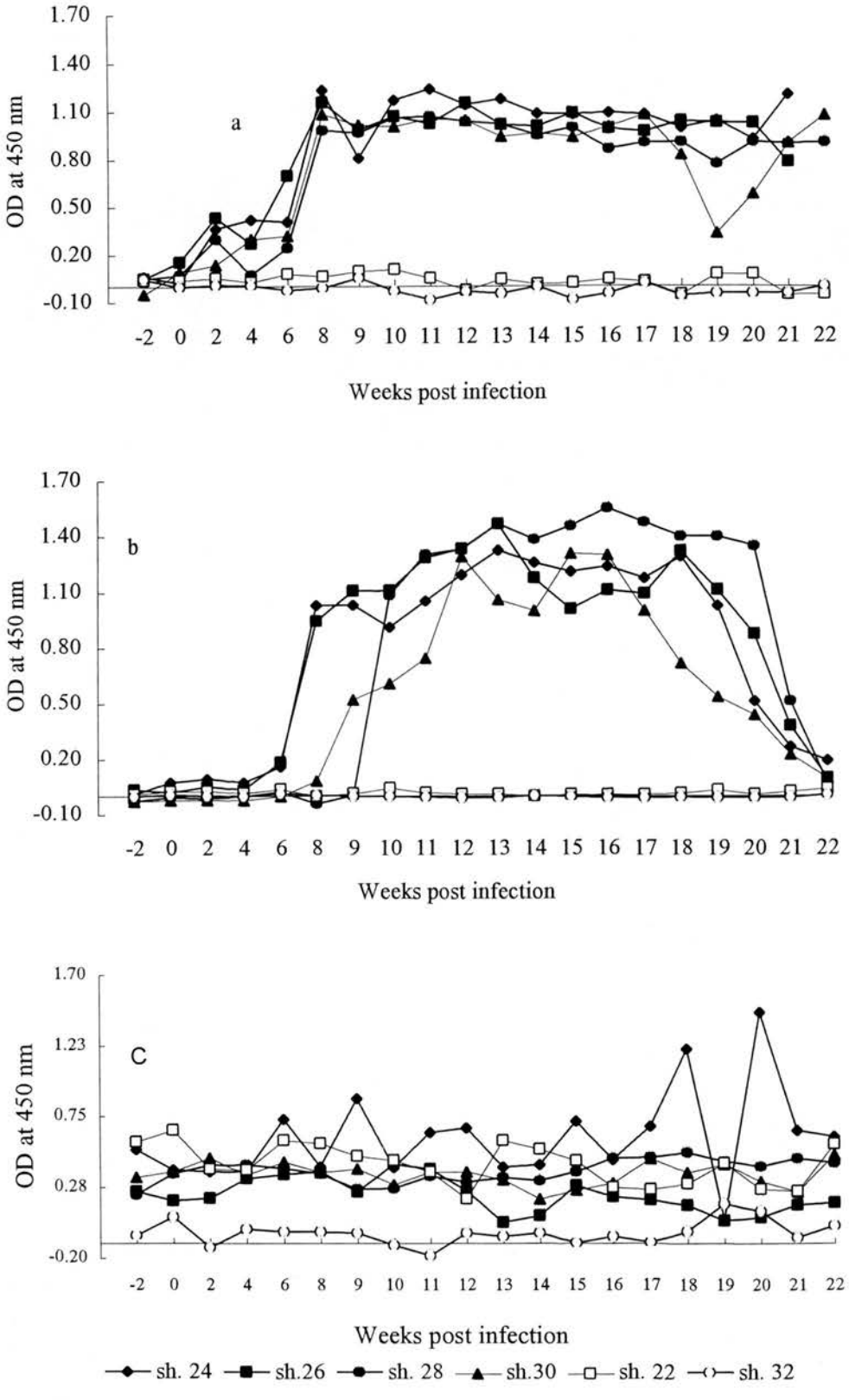


Figure 4.69: The ELISA OD (450 nm) for total Ig (a), IgG_1 (b) and IgM (c) responses of *F. gigantica* infected (24, 26, 28 and 30) sheep and uninfected (22 and 32) sheep to Fh-cathepsin

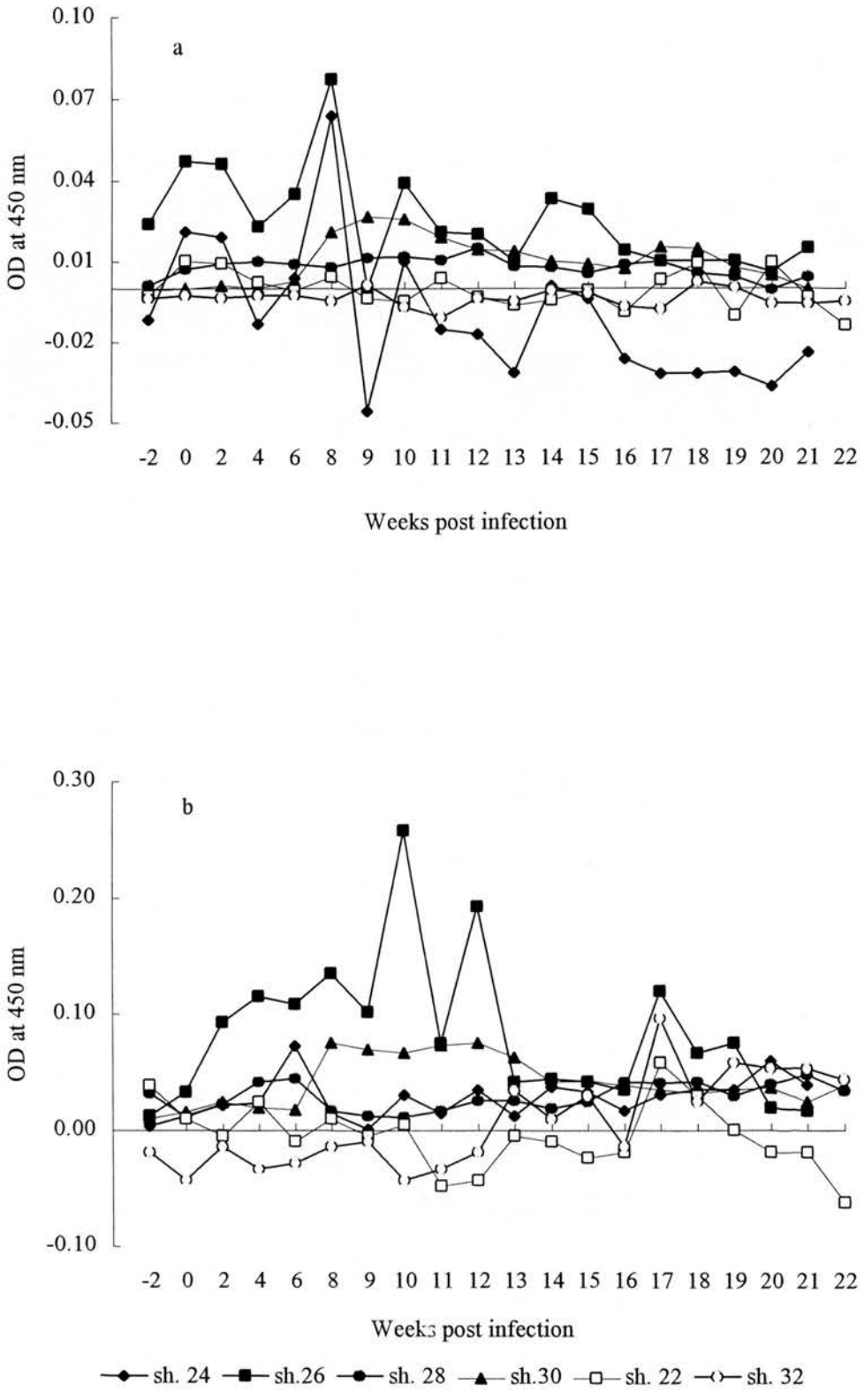


Figure 4.70. The ELISA OD (450 nm) for IgG_2 (a) and IgA (b) responses of *F. hepatica* infected sheep (24, 26, 28 and 30) and uninfected (22 and 32) sheep to Fh-cathepsin

4.3.4 Experiment 3: *F. Gigantica* (Kenyan Strain) Infection in Sheep

The antibody response to Fh-Cathepsin was easily detected in the sera of *F. gigantica* infected animals for total Ig and IgG_1. The subclasses IgM, IgG_2 and IgA recorded however lower values.

The infected sheep showed an increase in total Ig levels from 2 wpi. peaking at 11 wpi. Levels repressively fell thereafter but were still elevated by the end of experiment. (Figure 4.71a).

The IgG_1 isotype responses development were slowly peaked at 12-13 then started to reduce but by 21 wpi. however the infected sheep mean OD values were still raised (Figure 4.71b). T-Test analysis showed, however, that *Fasciola gigantica* infected sheep IgG1 response to Fh-cathepsin was significantly higher ($p < 0.005$) than the non infected sheep by 8 wpi., this is four weeks later than the IgG_1 response to Fg-E/S (Figure 4.51b).

The IgM response to Cathepsin-L protease in infected sheep was relatively poor, showed two peaks at 6 wpi. and the other at 20 wpi. (Figure 4.71c).

IgG_2 response to appeared biphasic starting from 2 wpi. with peak from 8-11 wpi. and a second peak occurring 16-17 wpi. (Figure 4.72a).

IgA responses were very poor and hardly visible at all (Figure 4.72b). The mean OD values *F. gigantica* infected animals appear to be above that of uninfected sub-group only weeks 2 wpi. The adjusted data in Appendix tables 4.91-95.

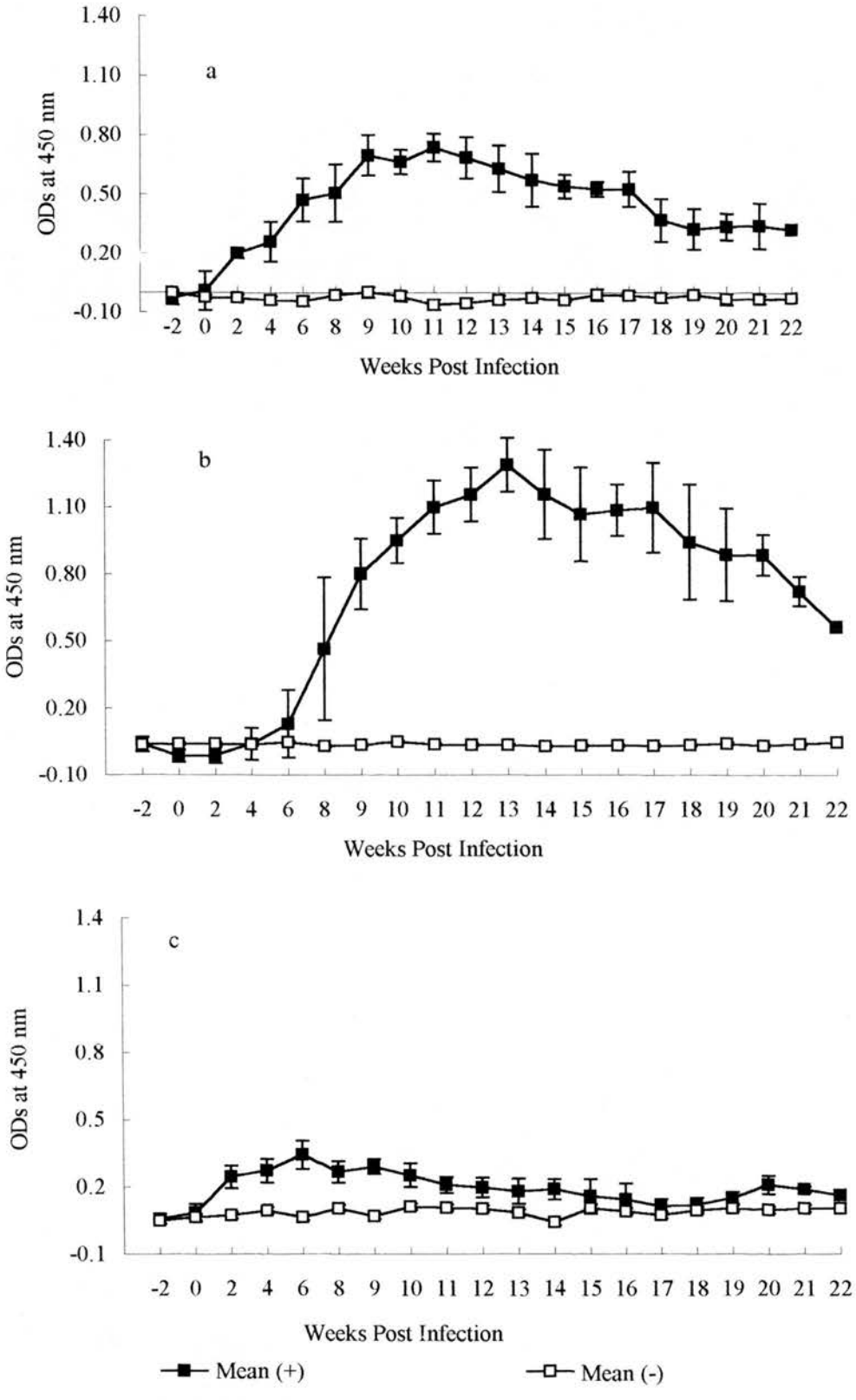


Figure 4.71. The ELISA ODs (450 nm) for mean ± SEM total Ig (a), IgG_1 (b) and IgM (c) responses of *F. gigantica* infected sheep (Mean (+) and uninfected control sheep (Mean (-) to Fh-cathepsin

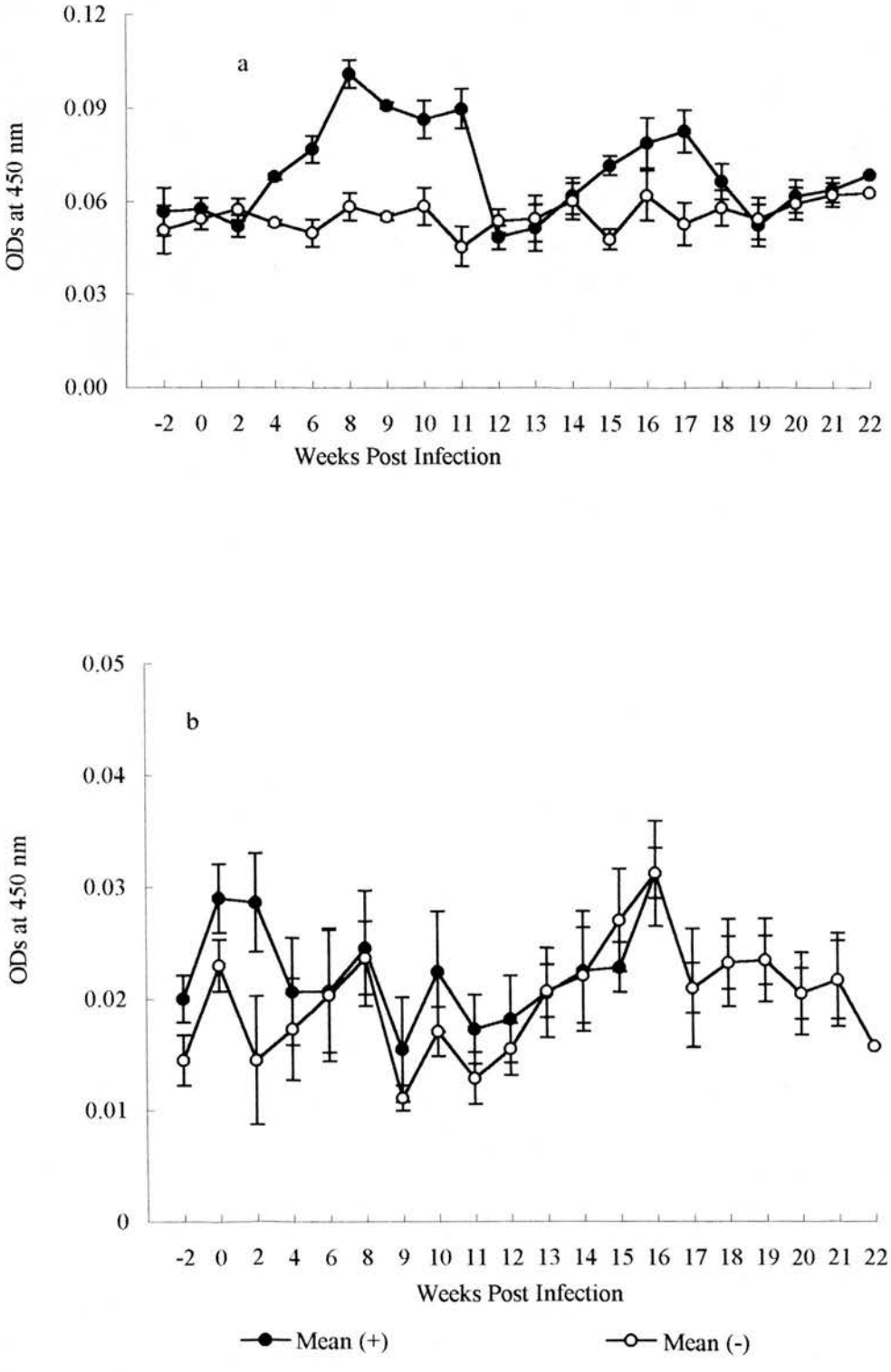


Figure 4.72: The ELISA OD (450 nm) for Mean ± SEM IgG_2 (a), IgA (b) responses of *F. gigantica* infected sheep (Mean (+)) and uninfected control sheep (Mean (-)) to Fh-cathepsin

4.3.5 Experiment 4: *F. Gigantica* (Kenyan Strain) Infection in Sheep

The antibody response to the Fh-cathepsin of the adult *F. hepatica* was easily detected in the sera of *F. gigantica* infected animals for total Ig, IgG1 IgM and IgG_2. IgA recorded however was very low.

The infected sheep showed a clear increase in total Ig levels by 8 wpi. The response peaked at 12 wpi. and remained high up to culling for sheep 23 and 25 and up to 20 wpi. for sheep 27 and 29 (Figure 4.73a).

The IgG_1 isotype responses to Cathepsin-L protease of infected sheep were not as strong as in *F. hepatica* and *F. gigantica* infected sheep in experiment four, however the OD's were markedly higher than the uninfected sheep 21 and 31 (Figure 4.73b).

The IgM response to Cathepsin-L protease in infected sheep was moderate (Figure 4.73c). The antibodies were present from 2 wpi. for all the infected sheep.

Only sheep 27 showed a clear IgG_2 response to Cathepsin-L protease (Figure 4.74a).

IgA responses were very poor and hardly visible at all. (Figure 4.74b).

The adjusted mean total Ig ELISA assay results and the isotype-specific antibody responses to Cathepsin-L protease of this experiment in Appendix tables 4.96-4.98.

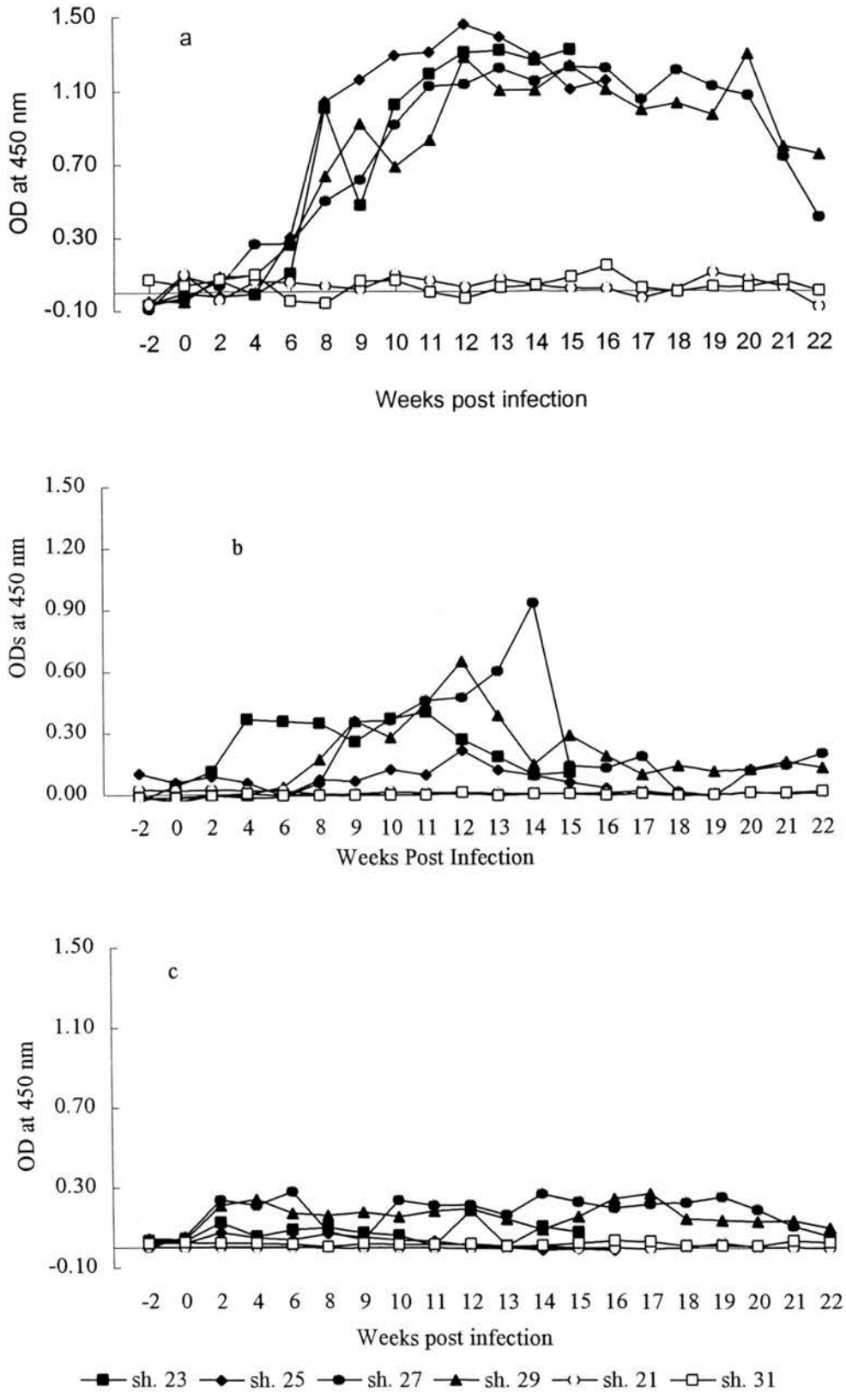


Figure. 4.73 The ELISA OD (450 nm) for total Ig (a), IgG_1 (b) and Ig M (c) responses of *F. gigantica* infected (23 ,25, 27, 29) and uninfected (21,31) sheep to Fh-cathepsin

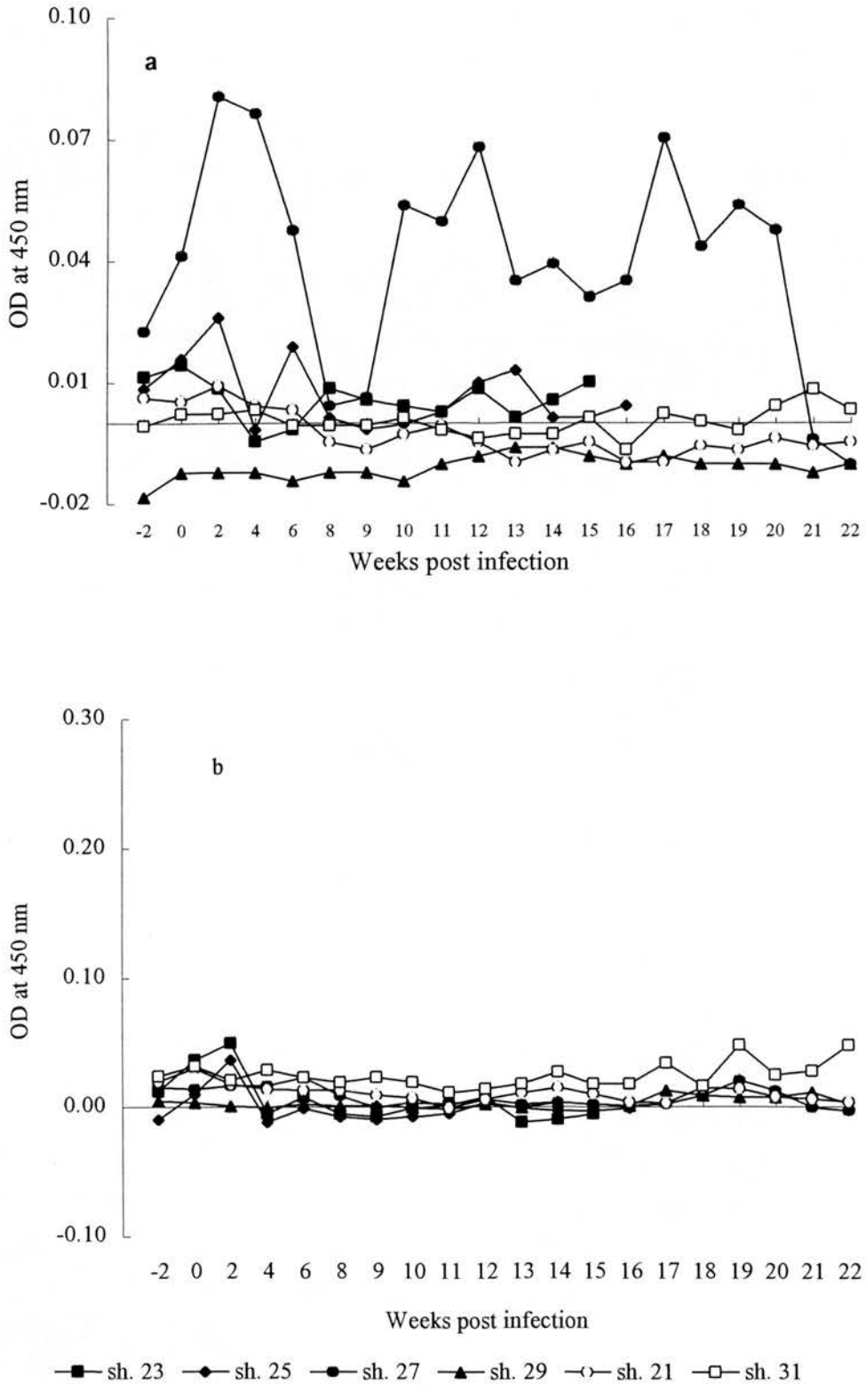


Figure 4.74: The ELISA OD (450 nm) for IgG_2 (a) and IgA (b) responses of *F. gigantica* infected sheep (23 ,25, 27 and 29) and uninfected (21and 31) sheep to Fh-cathepsin

4.3.6 Experiment 5: *F. Hepatica* (Peruvian Strain) Infection in Cattle

These cattle were either given primary infection as indicated or an infection and a challenge (Table 4.5 in section 4.1.5).

All the infected calves showed an increase in total Ig levels from +6-8 wpi. peaking by 9-10 wpi. and then gradually reducing. Antibody levels rose in calf 15c showed a second rise 5 weeks post challenge infection. This was not seen in calf 23c however the antibody remained higher until the end of the experiment (Figure 4.75a).

IgG_1 isotype responses were very pronounced. As with total Ig, the IgG1 response were detected 6-8 wpi. peaking by 9-10 wpi. The response following receiving challenge was also similar to that of total Ig (Figure 4.75b).

IgM levels followed an erratic pattern during the course of infection with a response first detected 4 wpi. and remained high throughout the experiment (Figure 4.75c).

Serum IgG_2 levels rose slowly from 9 wpi. increasing gradually however reduced in all the calves by the end of the experiment. The challenged infection did not appear to affect IgG_2 levels at all (Figure 4.76a).

IgA responses could be detected by 3-6 wpi. one animal, Calf 23c with a primary infection dose of 600 metacercariae had a particularly strong response. There was noticeable rise in IgA following secondary infection (Figure 4.76b).

The adjusted data is presented in appendix table 4.99-4.103.

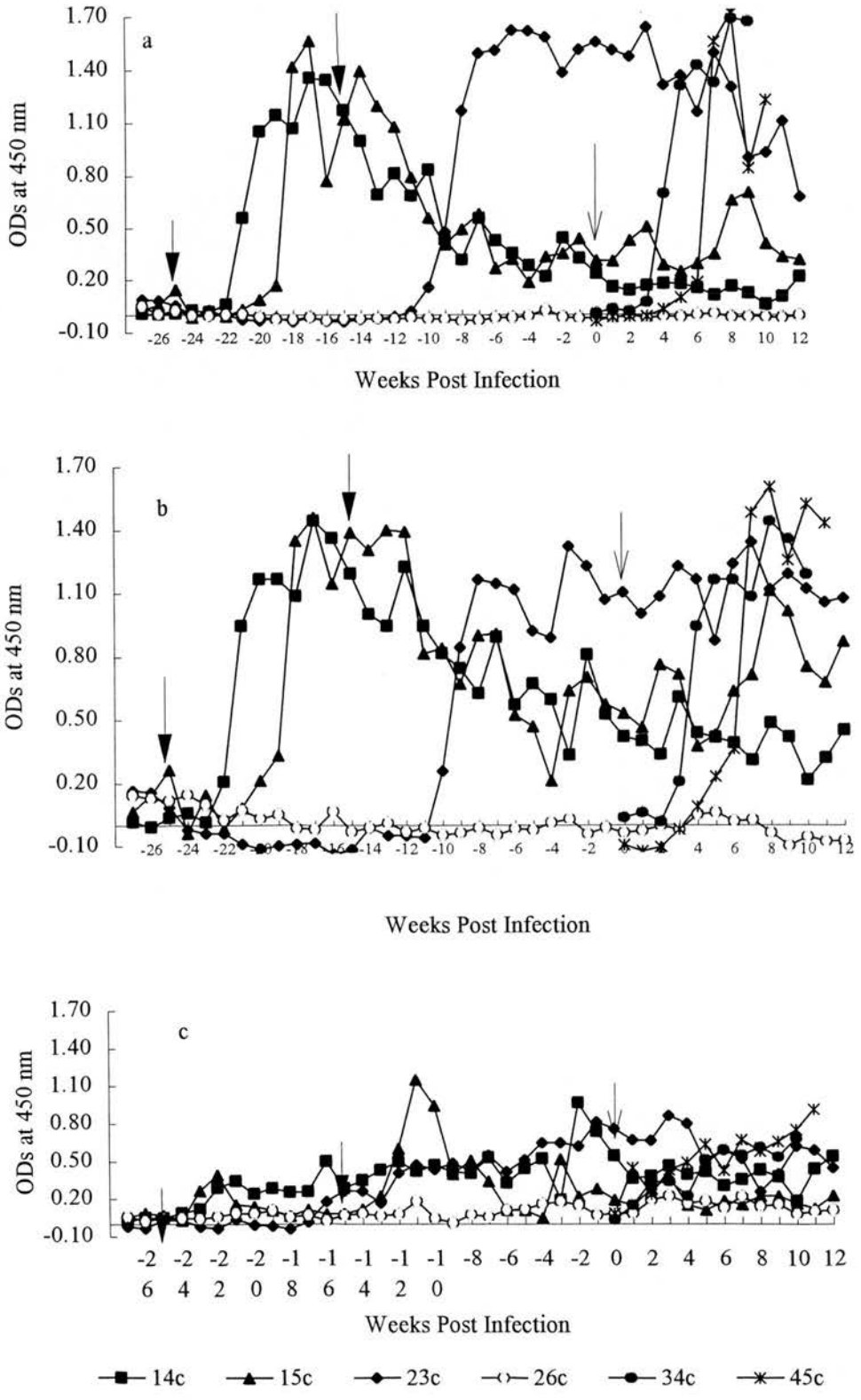


Figure 4.75: The ELISA OD (450 nm) for Total Ig (a), IgG_1 (b) and IgM (c) responses of *F. hepatica* infected calves 14, 15, 23, 34 and 45) and uninfecte control calves (26) to Fh-Cathepsin

Primary infection ⟹ Challenge infection

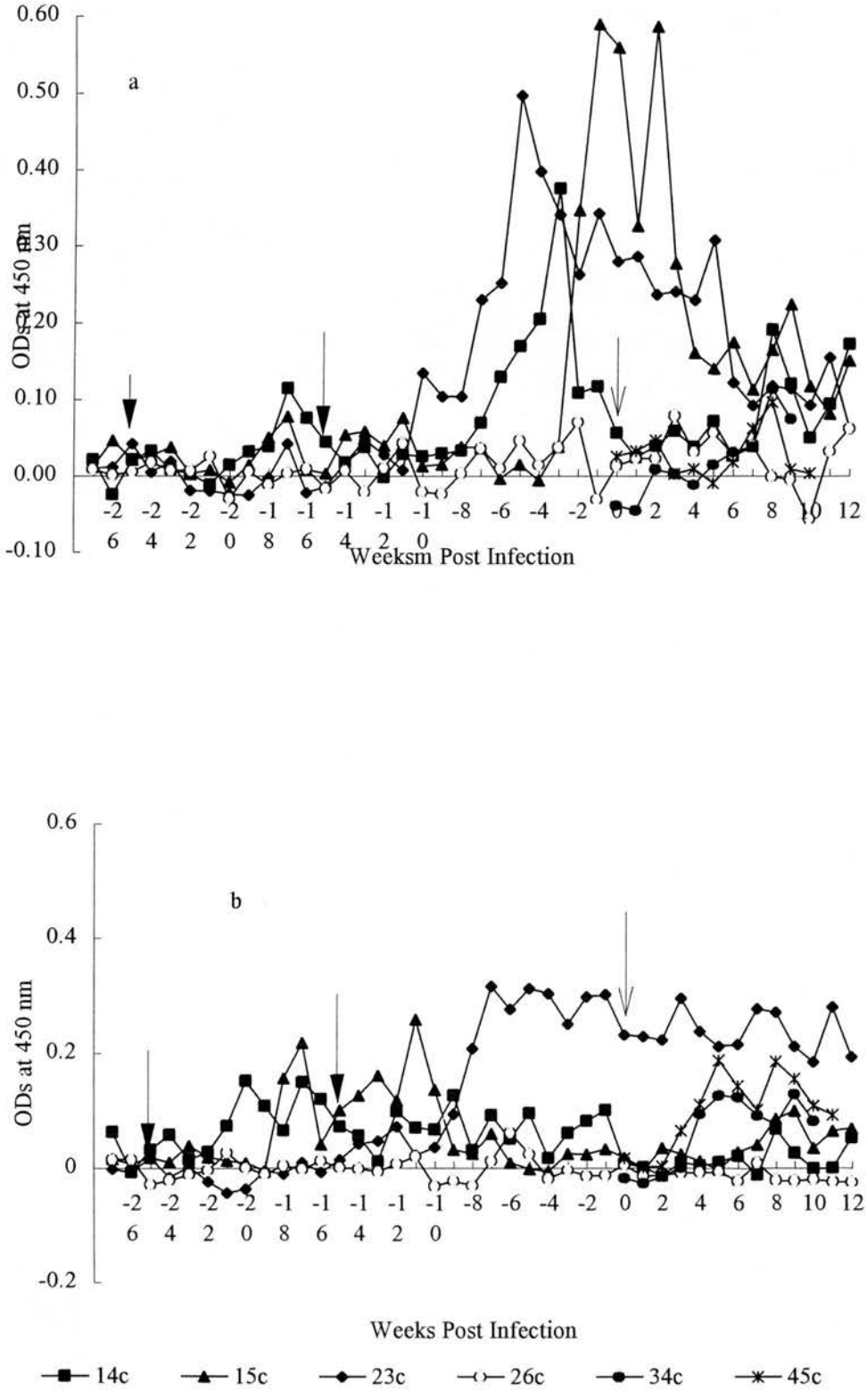


Figure 4.76: The ELISA OD (450 nm) for IgG_2 (a) and IgA (b) responses of *F. hepatica* infected calves (14c, 15c, 23c, 34c and 45c) and uninfected contr calf (26c) to Fh-Cathepsin. ——► Primary infection ——⇒ Challenge infection

4.3.7 Experiment 6: *F. Gigantica* (Kenyan Strain) Infection in Cattle

The polyclonal ELISA assay system was used to detect the total Ig and the isotype-specific antibody responses to Fh-Cathepsin by *F. gigantica* infected calves. Following infection, the antibodies were easily detected in the sera of infected calves for both total Ig and the four subclasses, IgG1, IgM, IgG_2 and IgA. However the kinetic of the antibody responses varied considerably (Figures 4.77a-4.78b).

Infected calves showed an increase in total Ig levels to Fh-Cathepsin from +7-9 wpi. peaking +15 wpi. and remaining high throughout the experiment. Although Calf 22 showed the poorest response (Figure 4.77a).

The kinetic of serum IgG_1 responses to Cathepsin-L protease showed a near similar pattern to those of total Ig but more stronger(Figure 4.77b).

IgM responses rose sharply by 3 wpi. with a much variable response thereafter (Figure 4.77c).

Interestingly the circulating IgG_2 antibody responses were very strong in all the calves. Calf 22 was the first (3 wpi.) to respond in contrast to total and IgG_1 responses (Figure 4.78a).

A gradual increases in IgA response was evident with increasing trend until the end of experiment (Figure 4.78b). All the data is presented in Appendix table 4.99-103.

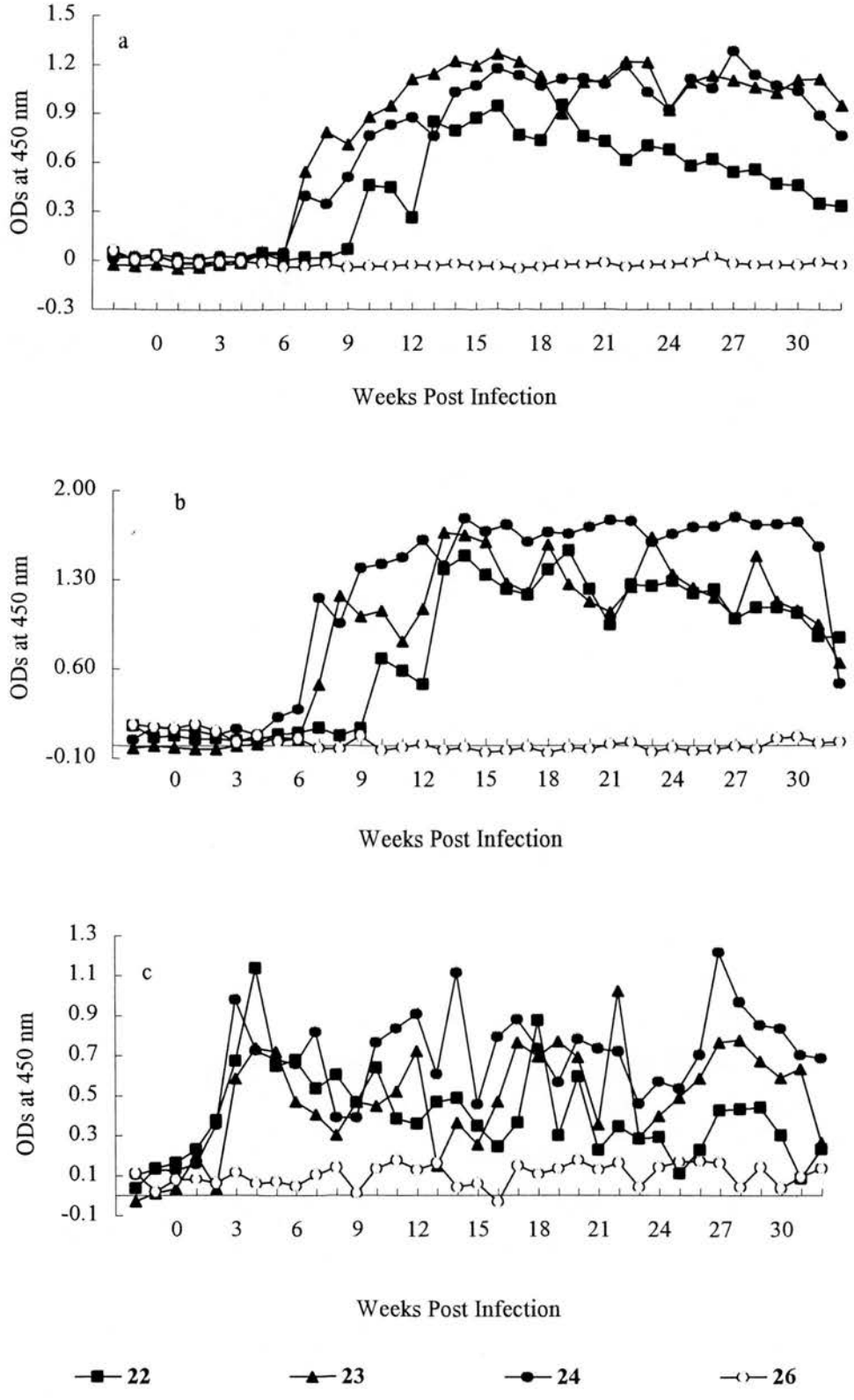


Figure 4.77: The ELISA OD (450 nm) for Total Ig (a), IgG_1 (b) and IgM (c) responses of *F. gigantica* infected calves (22, 23 and 24) and uninfected control (calf 26) to Fh-Cathepsin

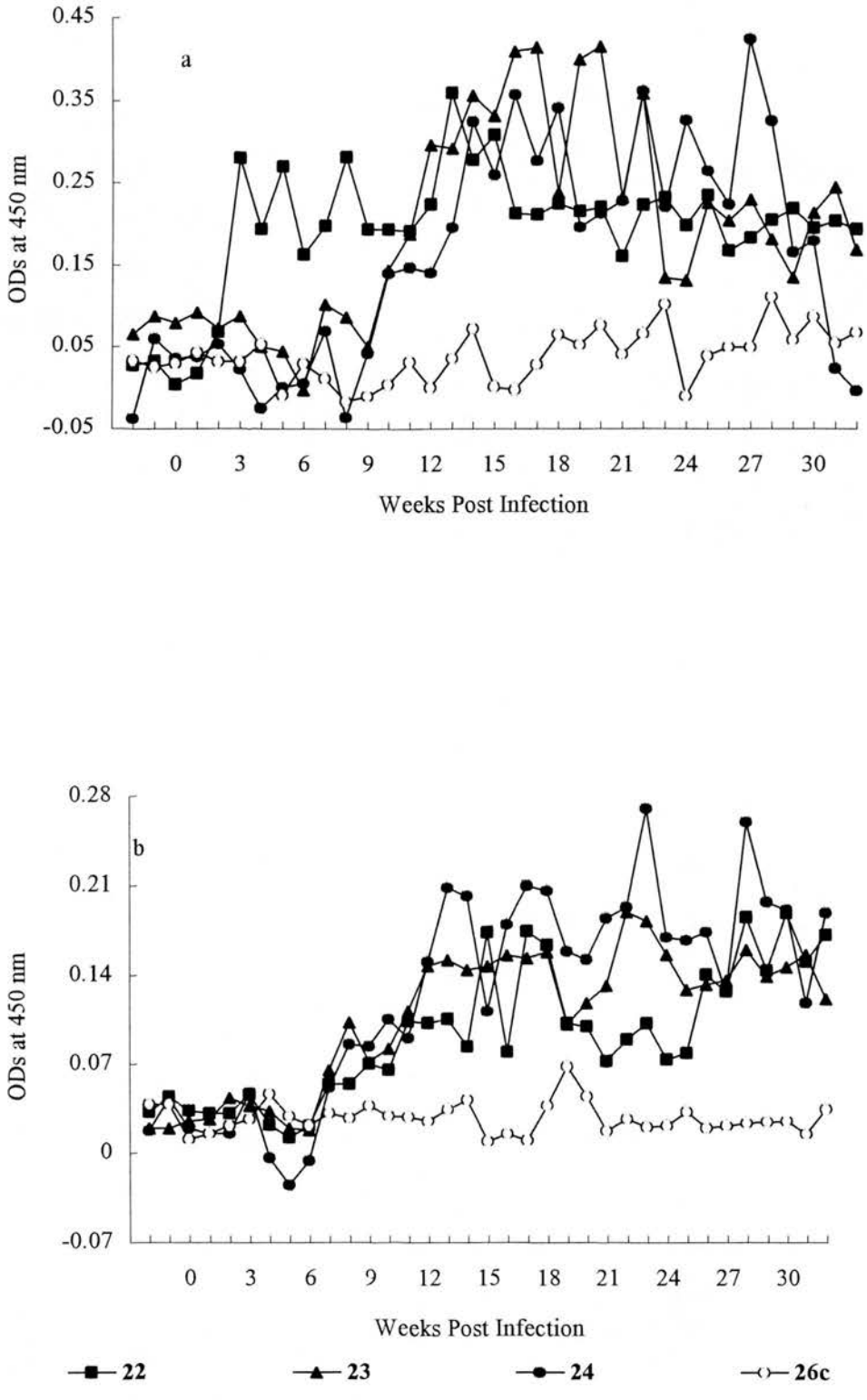


Figure 4.78: The ELISA OD (450 nm) for IgG_2 (a) and IgA (b) responses of *F. gigantica* infected calves (22, 23 and 24) and uninfected control calf (2 to Fh-Cathepsin

4.4 SERUM ANTIBODY RESPONSES AGAINST *F. HEPATICA* GLUTATHIONE S-TRANSFERASE (FH-GST)

4.4.1 Determination of Optimum Assay Condition by Titration.

The results for titrations to determine optimum antigen serum, monoclonal antibody and the conjugate dilutions are summarised in Tables 4.14-4.16. The antigen concentration to be used in the ELISA assay for each of the isotypes was determined by Fh-GST titration at 4, 2 and 1 μg/ml. The selected concentration was 1 μg/ml for all of the isotypes in either *F. hepatica* or *F. gigantica* infected sheep and cattle. Figures 4.79-4.86 are representative titrations for total Ig and IgG_1 in *F. hepatica* and *F. gigantica* infected sheep and cattle for the two detection systems, polyclonal antibody (Ig) and monoclonal antibody (IgG_2) detection systems. Full Data is represented in Appendix 4.104-113

Chequerboard titration for serum, monoclonal antibodies and conjugate were carried out by diluting these systems in blocking buffer using doubling serial dilution ranging from 4-1μg/ml (Antigen), 1:50-1:1600 (serum), 1:20-1:320 (monoclonal antibody) and 1:1000-1:32,000 (conjugate). Titrations were run in duplicates and the mean values calculated. The chosen antigen concentration, serum, monoclonal antibody and conjugate dilution were used in all subsequent sequential screenings. Dilution were selected on the basis of optimising the signal to background ratio. The two positive sera P1 and P2 were taken from *F. hepatica* or *F. gigantica* infected animals and corresponded to 8, 9 or 10 wpi (P1) and 21, 22 or 23 wpi (P2) for sheep and 7 wpi (P1) and 32 wpi (P2) for calves. These times post infection were chosen to

assess responses at the middle and at the end of the experimental period and to optimise the signal to background ratio..

Table 4.14 Sheep infected with *F. hepatica* or *F. gigantica*: Optimal dilutions of serum and conjugate for polyclonal antibody system using Glutathione S-Transferase from adult *F. hepatica* (Fh-GST) as antigen. Antigen concentration of 1μg/ml in all the sheep assays.

Assay	*F. hepatica*			*F. gigantica*		
	Fh-GST (μg/ml)	Serum	Conj.	Fh-GST (μg/ml)	Serum	Conj.
Total Ig	1	1:200	1:4000	1	1:200	1:4000
IgM	1	1:200	1:2000	1	1:200	1:2000

Table 4.15 Sheep infected with *F. hepatica* or *F. gigantica*: Optimal dilutions of serum, Monoclonal antibodies (McAb) and conjugate for monoclonal antibody system using Glutathione S-Transferase from adult *F. hepatica* (Fh-GST) as antigen. Antigen concentration of 1μg/ml in all the sheep assays.

Assay	*F. hepatica*				*F. gigantica*			
	Fh-GST (μg/ml)	Serum	McAb	Conj.	Fh-GST (μg/ml)	Serum	McAb	Conj.
IgG_1	1	1:200	1:40	1:4000	1	1:200	1:40	1:4000
IgG_2	1	1:50	1:20	1:1000	1	1:50	1:20	1:1000
IgA	1	1:50	1:20	1:1000	1	1:50	1:20	1:1000

Table 4.16 Cattle infected with *F. hepatica* or *F. gigantica*: Optimal dilutions of serum and conjugate for polyclonal antibody system using Glutathione S-Transferase from adult *F. hepatica* (Fh-GST) as antigen. Antigen concentration of 1μg/ml in all the cattle assays.

Assay	*F. hepatica*			*F. gigantica*		
Assay	Fh-GST (μg/ml)	Serum	Conj.	Fh-GST (μg/ml)	Serum	Conj.
Total Ig	1	1:200	1:4000	1	1:200	1:4000
IgG_1	1	1:200	1:4000	1	1:200	1:4000
IgM	1	1:200	1:2000	1	1:200	1:2000
IgG_2	1	1:50	1:1000	1	1:50	1:1000
IgA	1	1:50	1:1000	1	1:50	1:1000

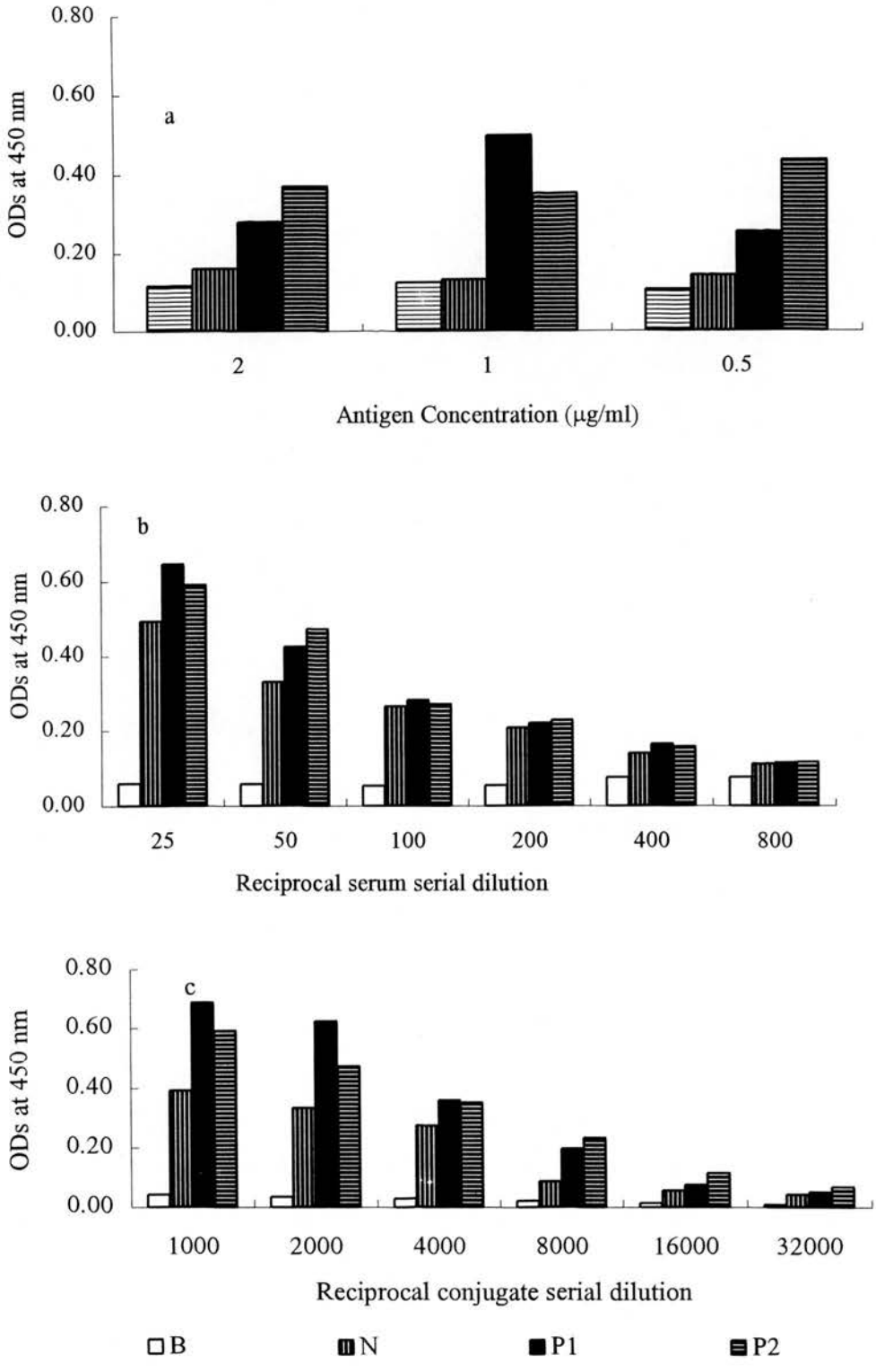


Figure 4.79: Antigen (FhGST) (a), Serum (b) and Conjugate (c) titration for total Ig for *F. hepatica* infected and uninfected control sheep showing the mean ELISA (450nm) values obtained for diluent (B), negative (N) positive (P1) and positive (P2)

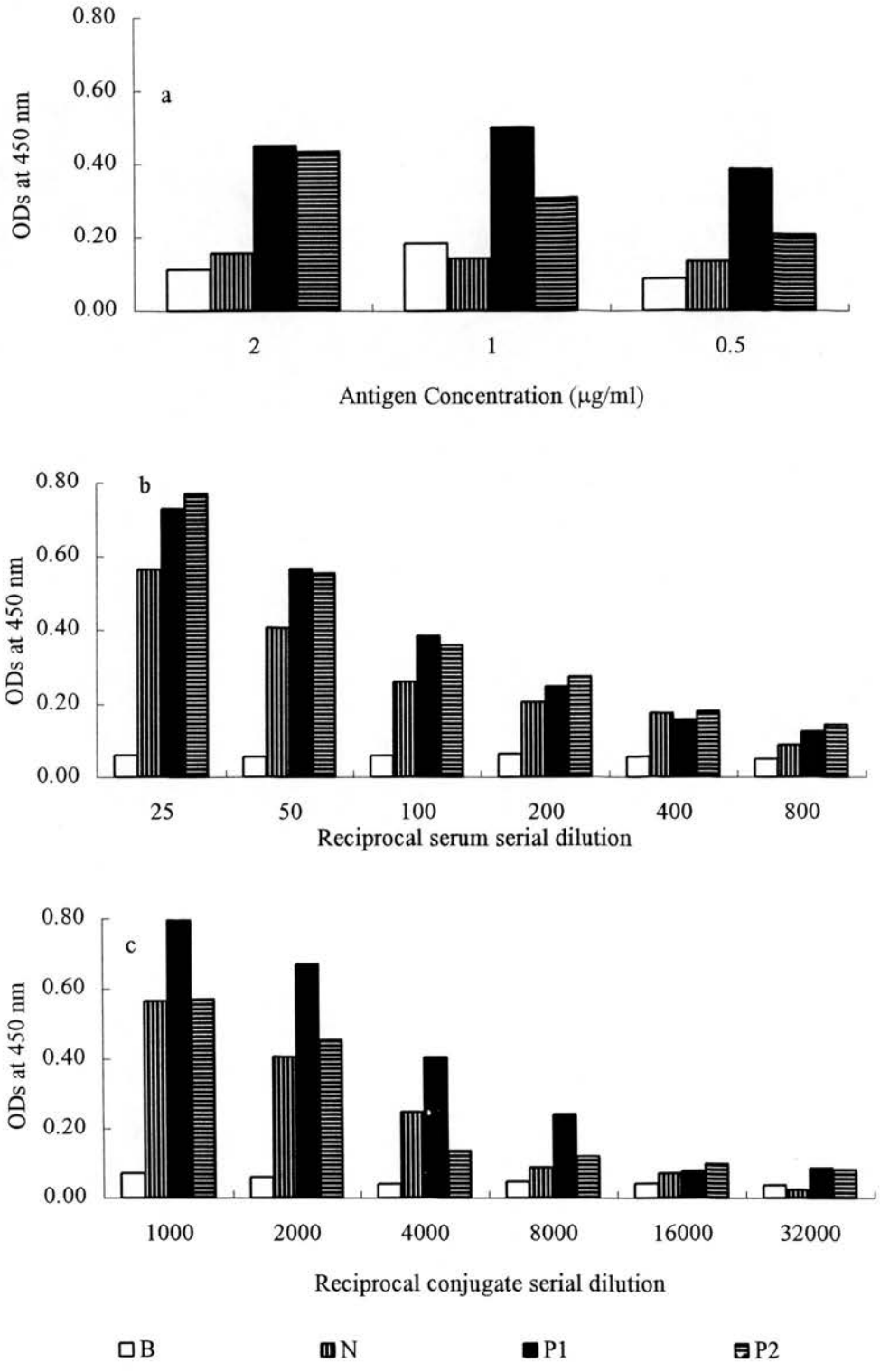


Figure 4.80: Antigen (FhGST) (a), Serum (b) and Conjugate (c) titration for total Ig for *F. hepatica* infected and uninfected control sheep showing the mean ELISA (450 nm) values obtained for diluent (B), negative (N) positive (P1) and positive (P2)

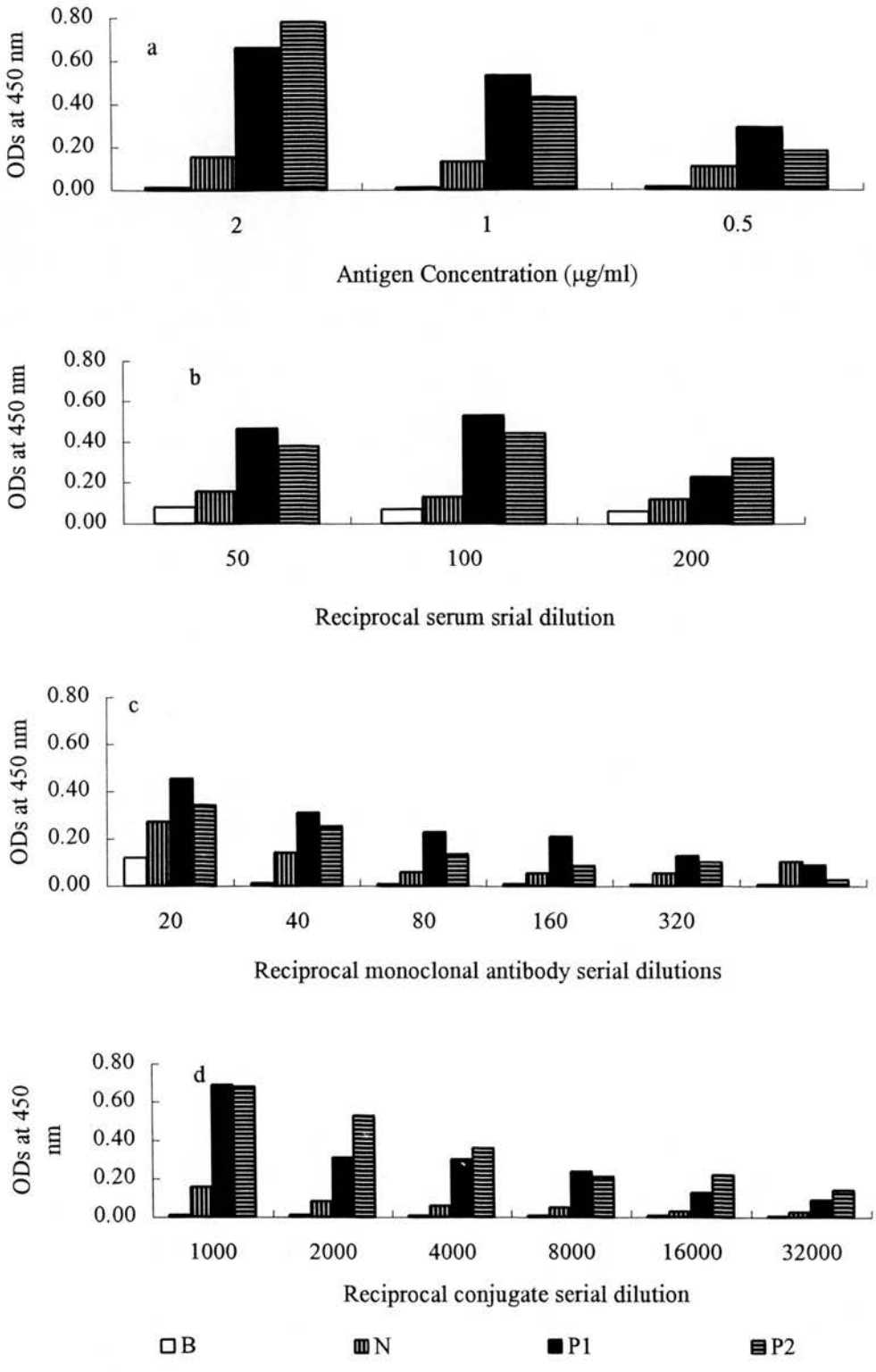


Figure 4.81: Antigen (Fh-GST) (a) serum (b), monoclonal (c) and conjugate (d) titration for total Ig for *F. hepatica* infected and uninfected control sheep showing the mean ELISA values obtained for diluent (B) negative (N) positive (P1) and positive (P2)

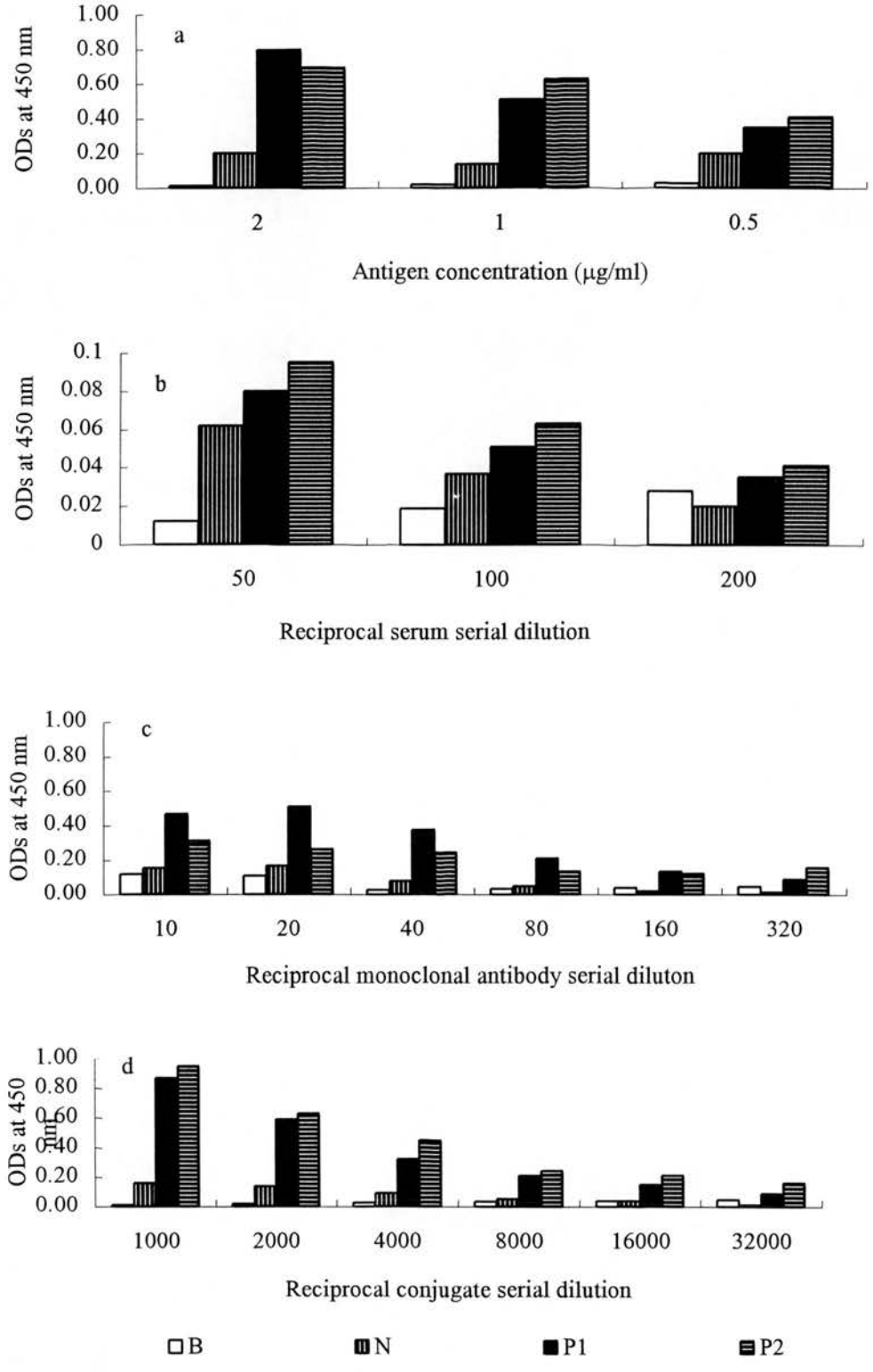


Figure 4.82: Antigen (Fh-GST) (a) Serum (b), Monoclonal (c) and Conjugate (d) titration for total Ig for *F. gigantica* infected sheep and uninfected control sheep showing mean ELISA (450 nm) values obtained for diluent (B) negative (N) positive (P1) and positive (P2)

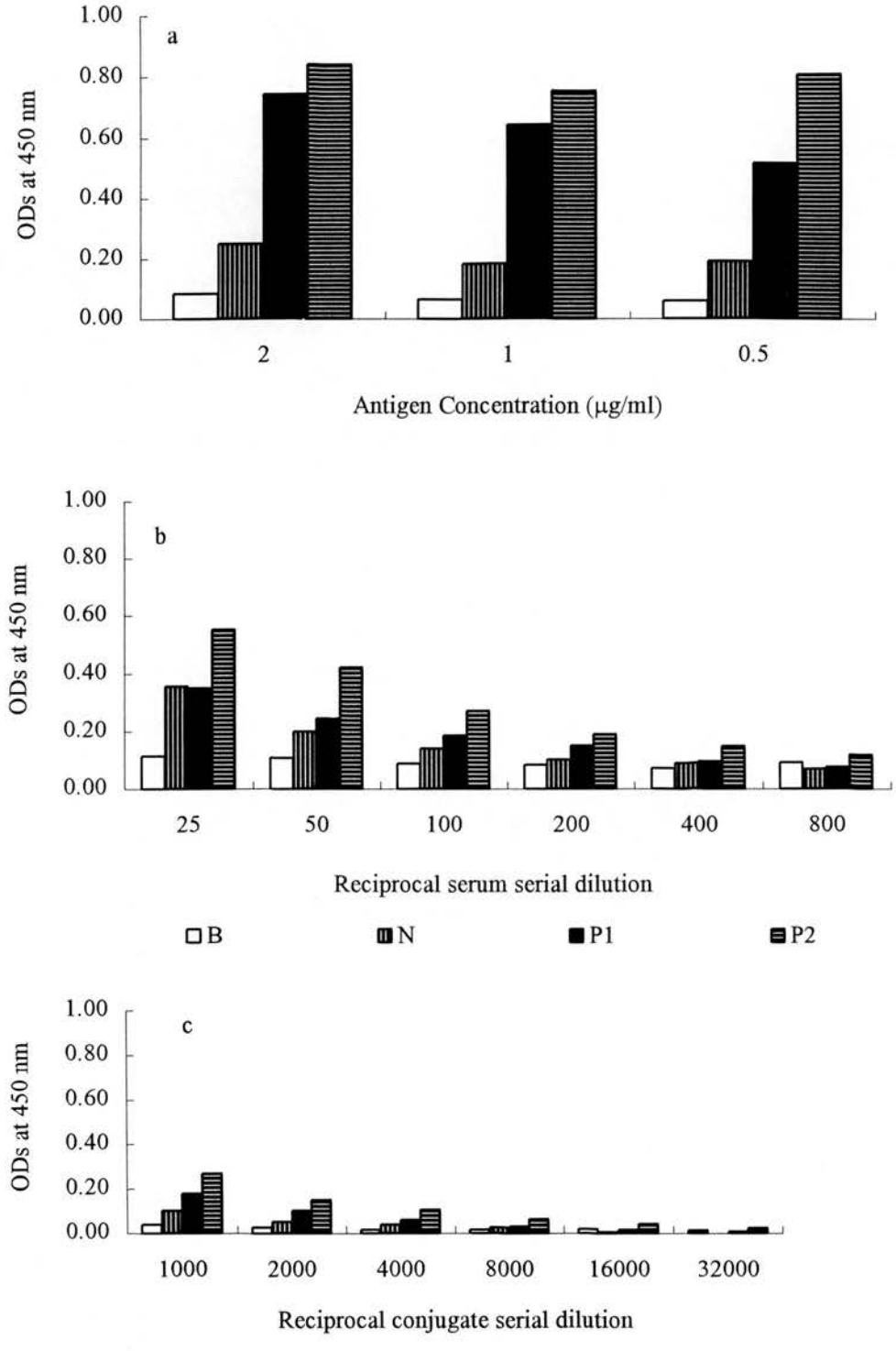


Figure 4.83: Antigen (Fh-GST) (a) serum (b) and conjugate (c) titration for total Ig for *F. hepatica* infected cattle and uninfected control cattle showing mean ELISA (450 nm) values obtained for diluent (B) negative (N) positive (P1) and positive (P2) negative (N) positive (P1) and positive (P2)

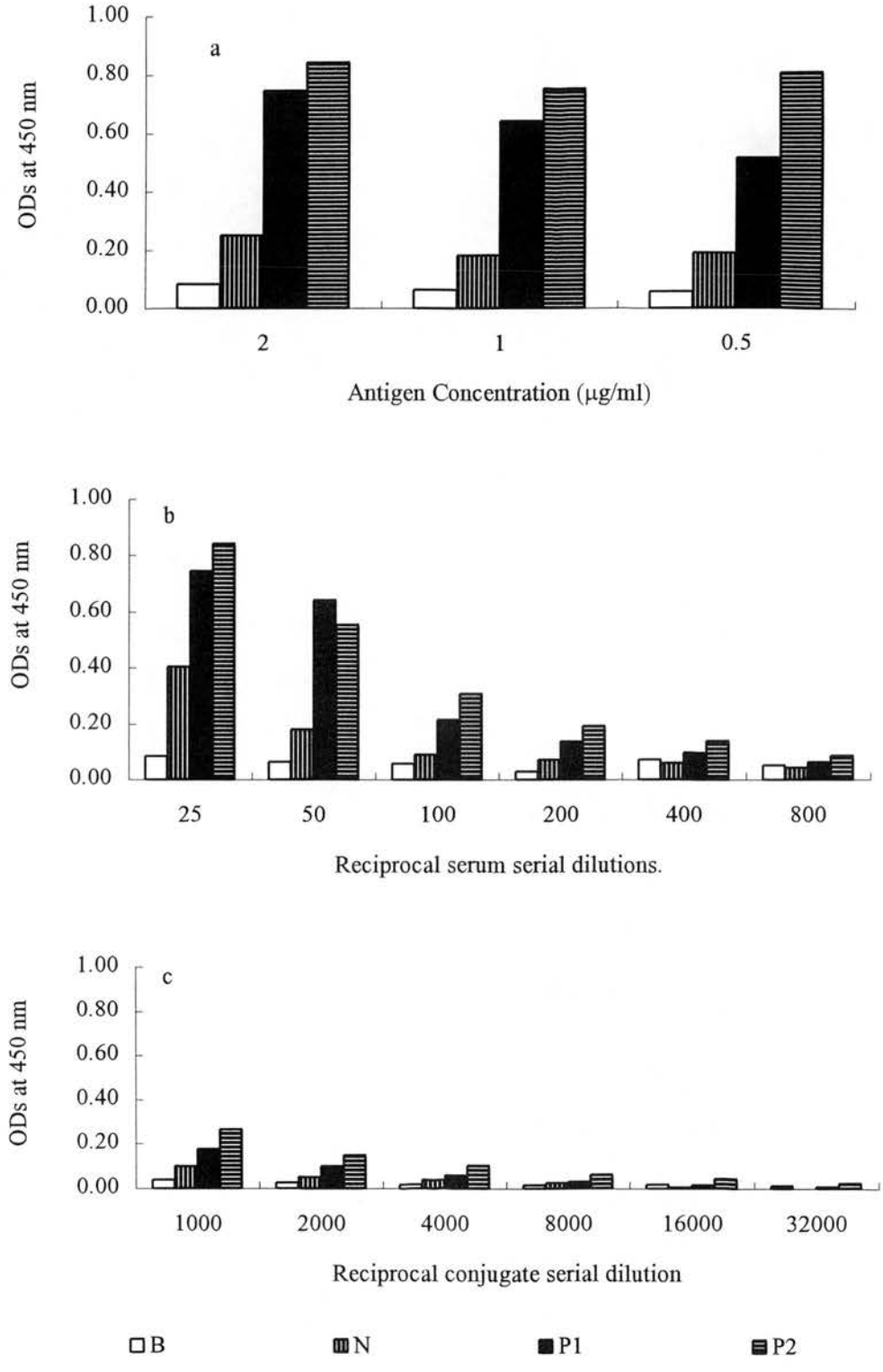


Figure 4.84: Antigen (FhGST) (a), serum (b) and conjugate (c) titration for total Ig for *F. gigantica* infected and uninfected cattle showing mean ELISA (450 nm) values obtained for diluent (B) negative (N) positive (P1) and positive (P2)

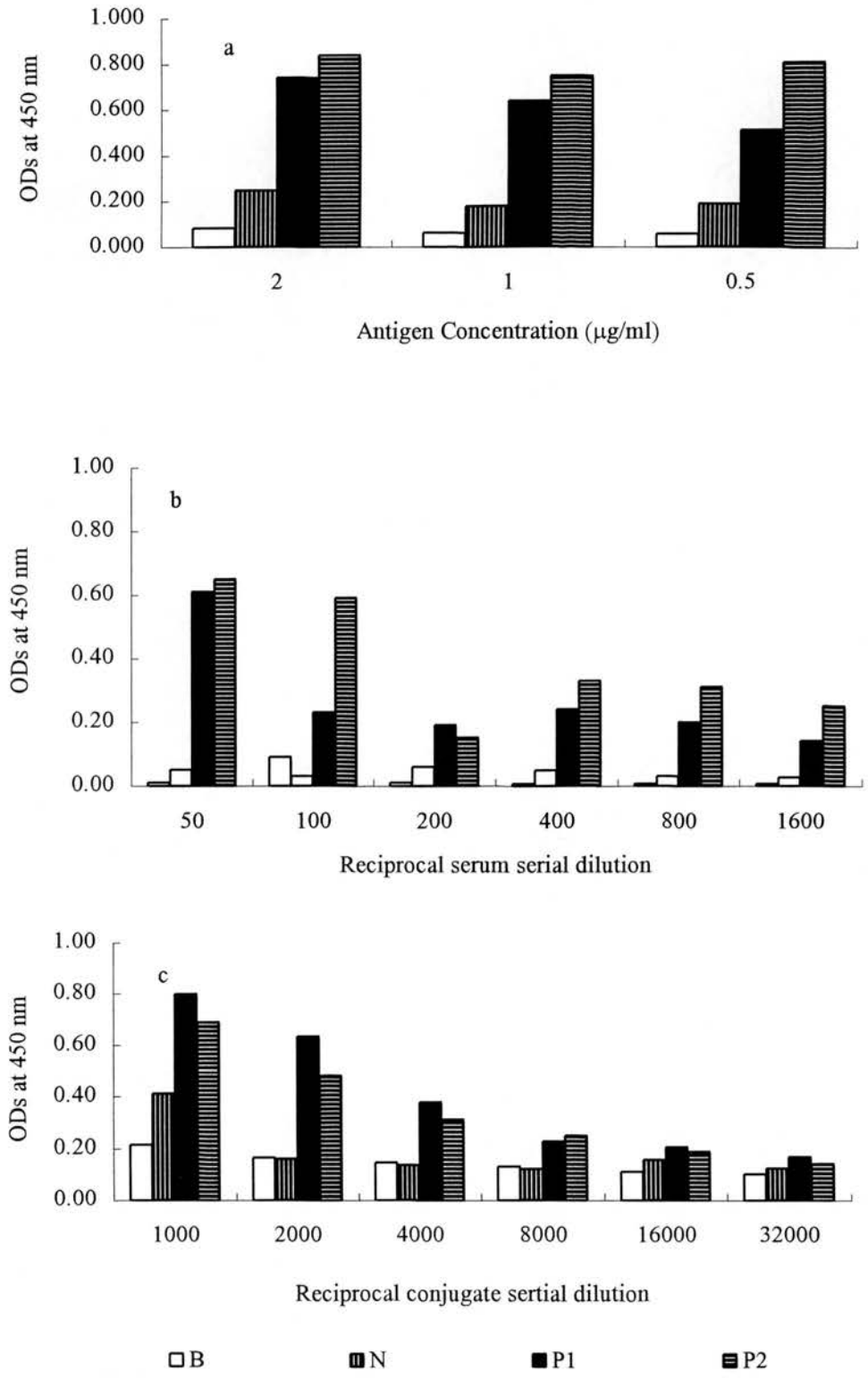


Figure 4.85: Antigen (FhGST) (a), serum (b) and conjugate (c) titration for IgG_1 for *F. hepatica* infected and uninfected control cattle showing the mean ELISA (450 nm) values obtained for diluent (B), negative (N) positive (P1) and positive (P2)

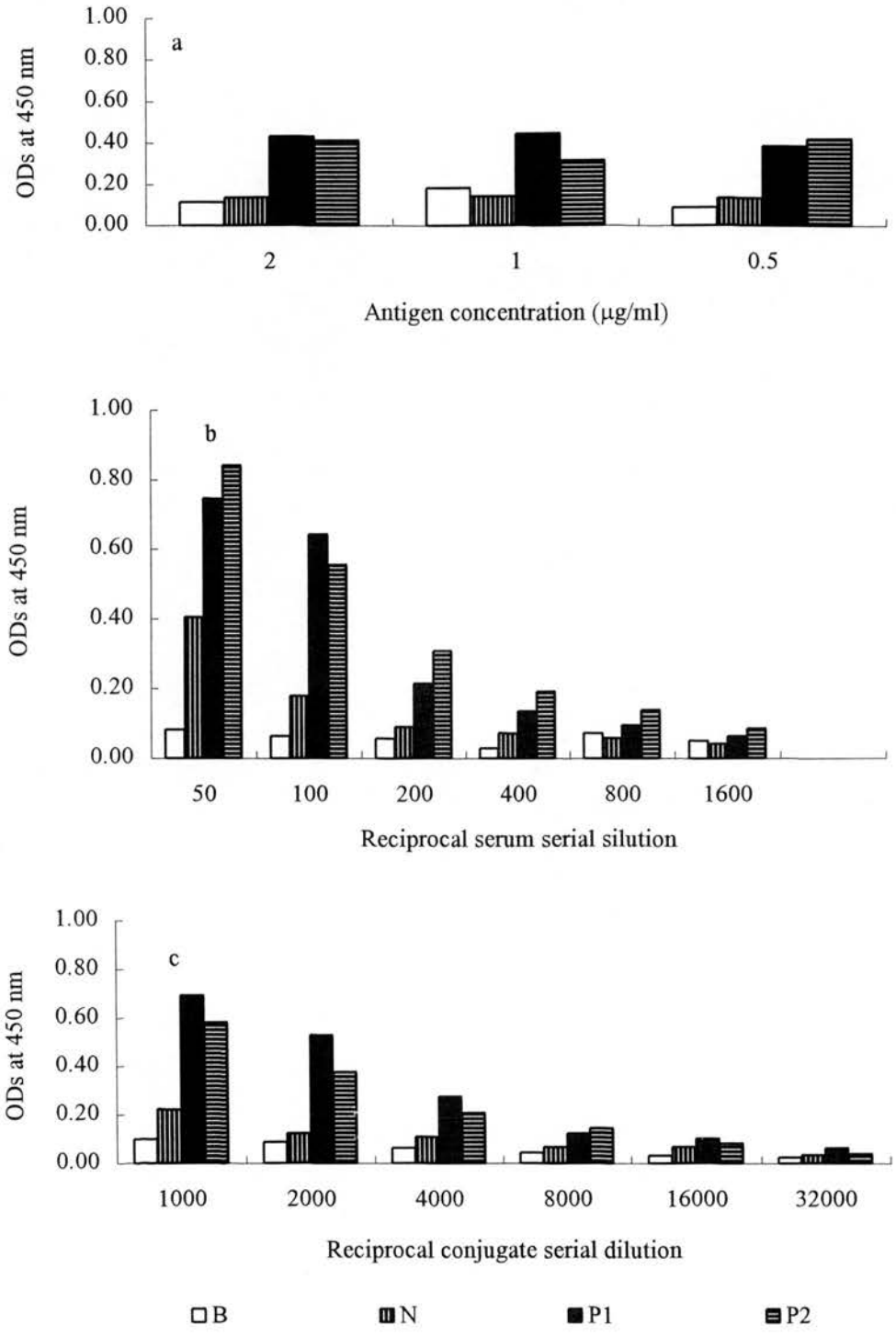


Figure 4.86: Antigen (FhGST) (a) serum (b) and conjugate (c) titrations for IgG_1 for *F. gigantica* infected and uninfected control cattle showing mean ELISA (450 nm) values obtained for diluent (B) negative (N) positive (P1) and positive (P2)

4.4.2 Experiment 1: *F. Hepatica* (Peruvian And British Strain) Infection in Sheep

Following primary infection, serum total Ig, IgG_1, IgM, IgG_2 and IgA antibodies responses to Fh-GST were detected in the *F. hepatica* infected sheep. There was however a moderate IgG2 and IgA response.

All the infected sheep showed a late increase in total Ig levels with a clear increase by 6 wpi. peaking at 11 wpi. Sheep 6 with ODs above 0.7 nm at peak response (Figure 4.87a).

There were sharp and early, 2-3 wpi., IgG_1 isotype responses to Fh-GST by sheep 7, 9 and 10. There was no response by sheep 5 and little in sheep 6. as shown in Figure 4.87b.

A variable IgM response was clearly noticeable in sheep 6, 7, 9 and 10 but as with IgG1 sheep 5 did not respond (Figure 4.87c).

A delayed sera IgG_2 response to Fh-GST was recorded by sheep 6 and 5. The rest of infected sheep were no different to uninfected control sheep 6 (Figure 4.88a).

The only clear IgA response to Fh-GST was noticed in sheep 9 wpi., there is however some mild response by the rest of the infected sheep of this group as shown in Figure 4.88b. Data is represented in appendix tables 4.114-116.

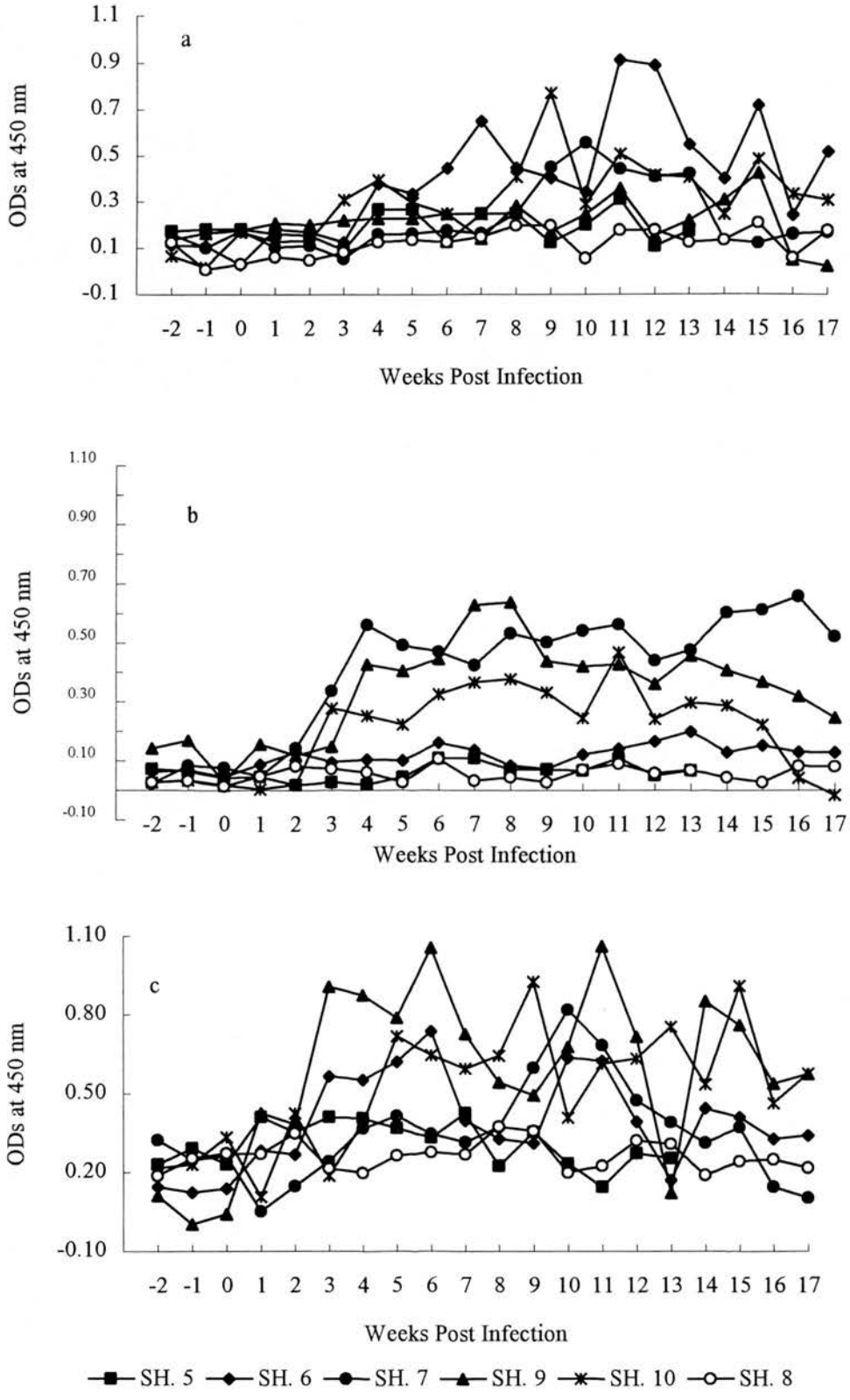


Figure 4.87: The ELISA OD (450 nm) values for serum total Ig (a), IgG_1 (b) and IgM (c) responses of *F. hepatica* infected sheep (5, 6, 7, 9 and 10) and uninfected control sheep (8) to FhGST

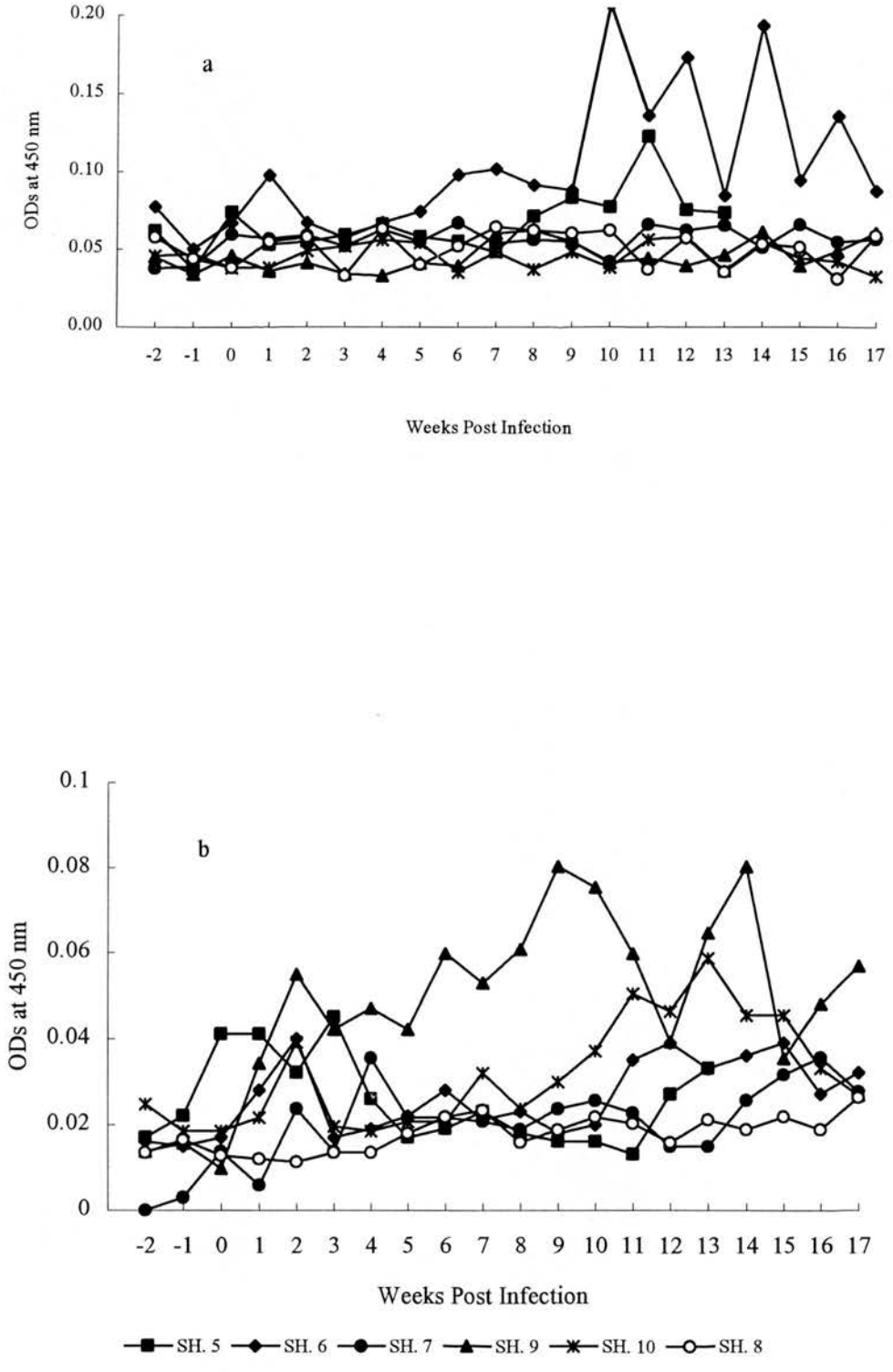


Figure 4.88: The ELISA OD (450 nm) values for serum IgG_2 (a) and IgA (b) responses of *F. hepatica* infected sheep (5, 6, 7, 9 and 10) and uninfected control sheep 8 to FhGST

4.4.3 Experiment 2: *F. hepatica* (British strain) Infection in Sheep

Antibody response to Fh-GST was detected in these *F. hepatica* infected sheep. The total Ig and IgM responses were greatest while IgG_1, IgG_2 and IgA were questionable, Figure 4.89a-4.90b. The adjusted data to each sheep are presented in Appendix Tables 4.117-119.

The infected sheep showed an early total Ig response to Fh-GST and the levels remained high throughout the experiment. IgG_1 isotype responses were only clear in sheep 26 and 30. The rest of the infected sheep did not show clear response.

The IgM responses was early and strong in all the infected sheep with peaks at 8-11 wpi. By 12 wpi. the OD values started to drop. Sheep 30 recorded lower values than the rest of the infected sheep. The IgG_2 and IgA response to Fh-GST by infected sheep was indistinguishable from the uninfected controls.

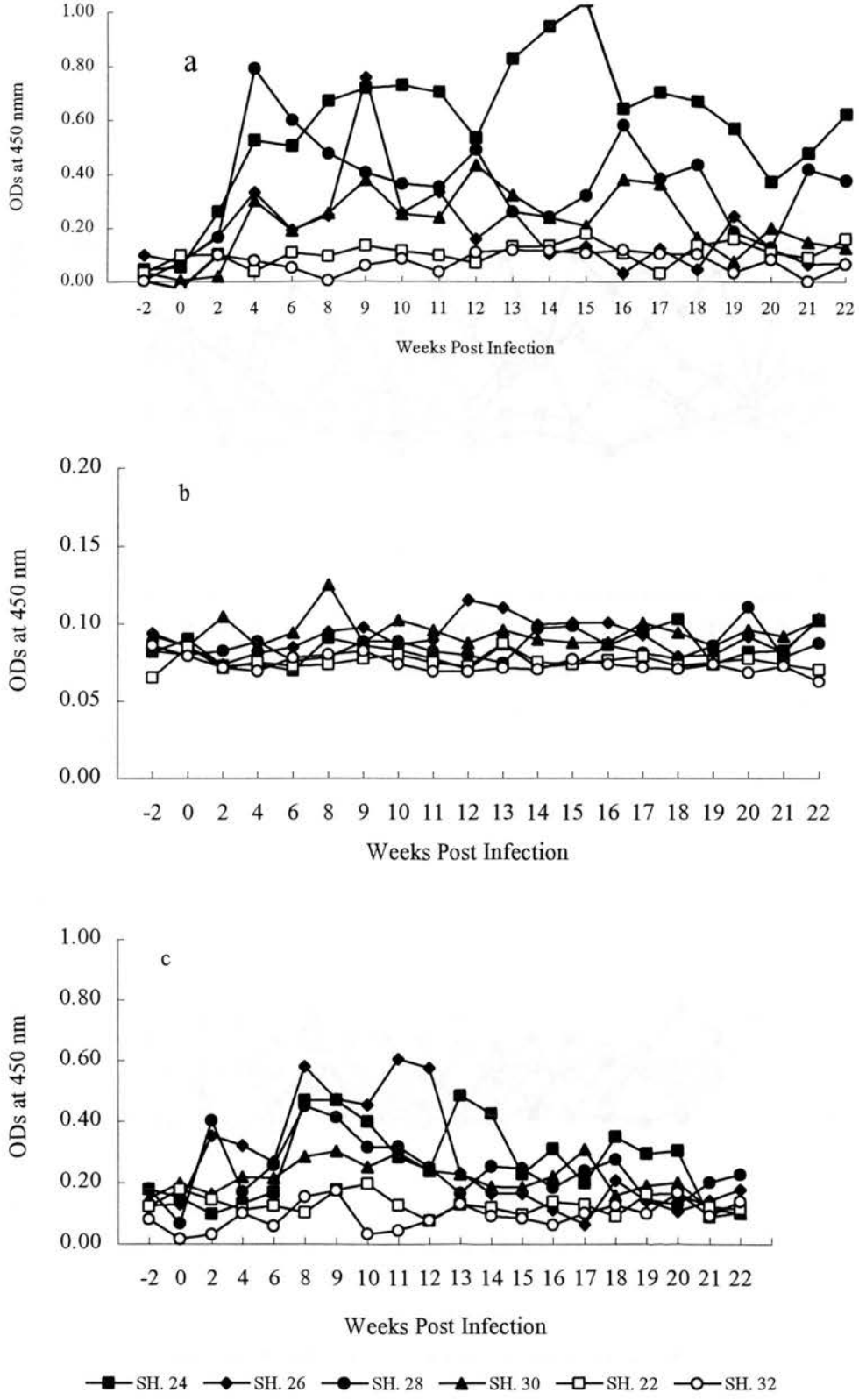


Figure 4.89: The ELISA OD (450 nm) values for serum total Ig (a), IgG_1 (b) and IgM (c) responses of *F. hepatica* infected sheep (24, 26, 28 and 30) and uninfected control sheep (22 and 32) to FhGST

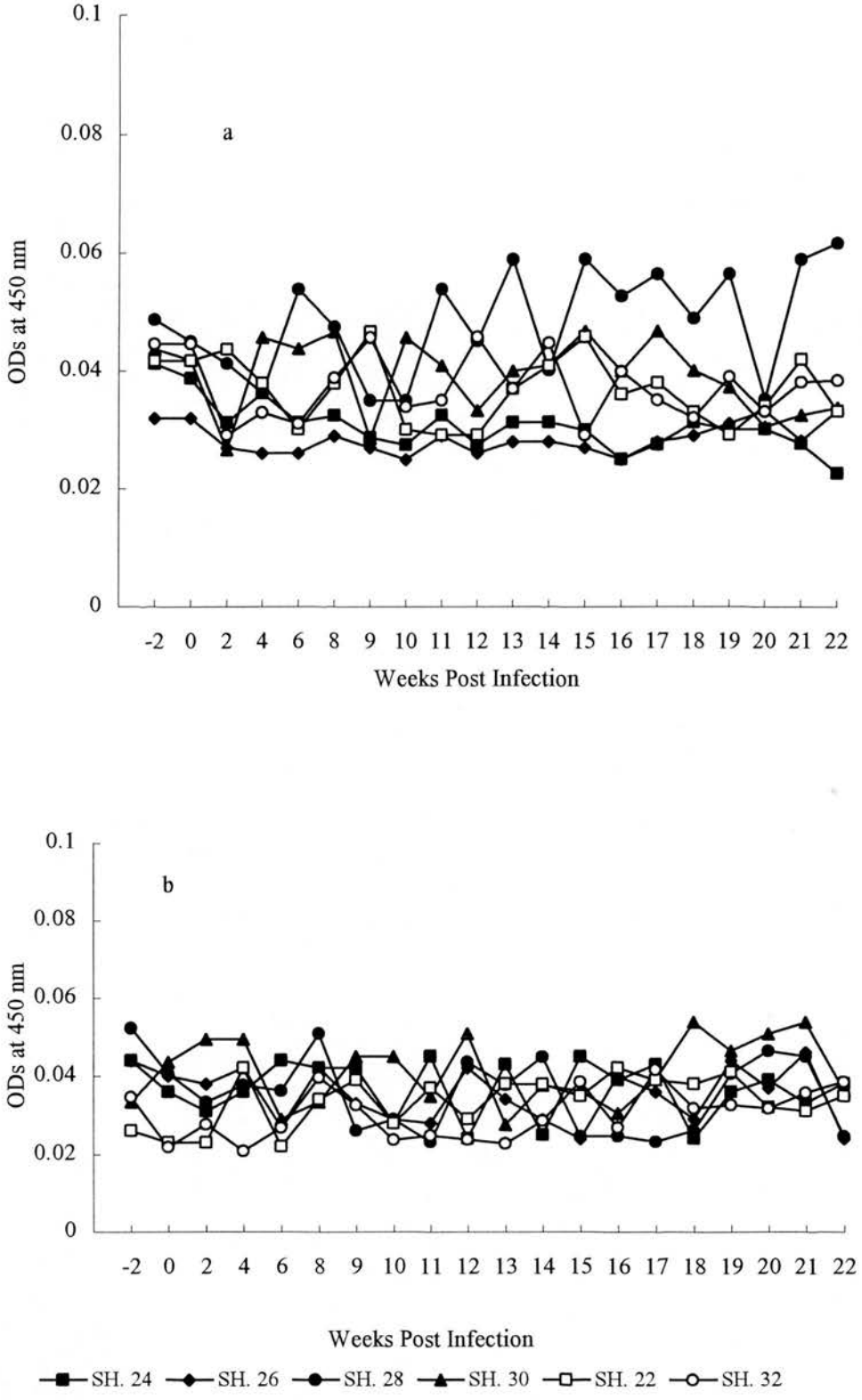


Figure 4.90: The ELISA OD (450 nm) values for serum IgG_2 (a) and IgA (b) responses of *F. hepatica* infected sheep 24, 26, 28 and 30 and uninfected control sheep 22 and 32 to FhGST

4.4.4 Experiment 3: *F. gigantica* (Kenya Strain) Infection in Sheep

The total Ig, IgM and IgG_1 antibody response to Fh-GST was easily detected in the sera of *F. gigantica* infected sheep. The IgG_2 and IgA responses, however were very poor. The mean antibody responses of *F. gigantica* infected sheep 11, 12, 13, 14, and 15 and uninfected control sheep 16, 17, 18 and 19 are presented in Figures 4.91a-4.92b

The infected sheep showed a very early (2 wpi.) total Ig response to Fh-GST. The mean OD levels remained high throughout the 22 weeks of infection with slight reduction (Figure 4.91a).

The IgG_1 isotype responses to Fh-GST was first noted in the *F. gigantica* infected sheep of this group after 2 wpi. with peak occurring at 4-9 wpi. The response slowly and steadily started to reduce but by 21 wpi. the infected sheep mean OD values were still high higher than the control (Figures 4.91b)..

The IgM response to Fh-GST in infected sheep was early and rose sharply in the first week of infection peaking at 10 wpi. The antibody response declined slowly after 11 wpi. There was a lot of variation within the individual infected sheep (Figures 4.91c)..

A mild IgG_2 response was noted from 9 wpi. The mean OD values of infected sheep were above the uninfected up to 17 wpi. (Figure 4.92a).

The IgA response to Fh-GST was slight but the mean OD values *F. gigantica* infected sheep were above that of uninfected controls between 4 wpi. and 17 wpi. (Figure 4.92b). Adjusted data is presented in Appendix tables 4.120-124.

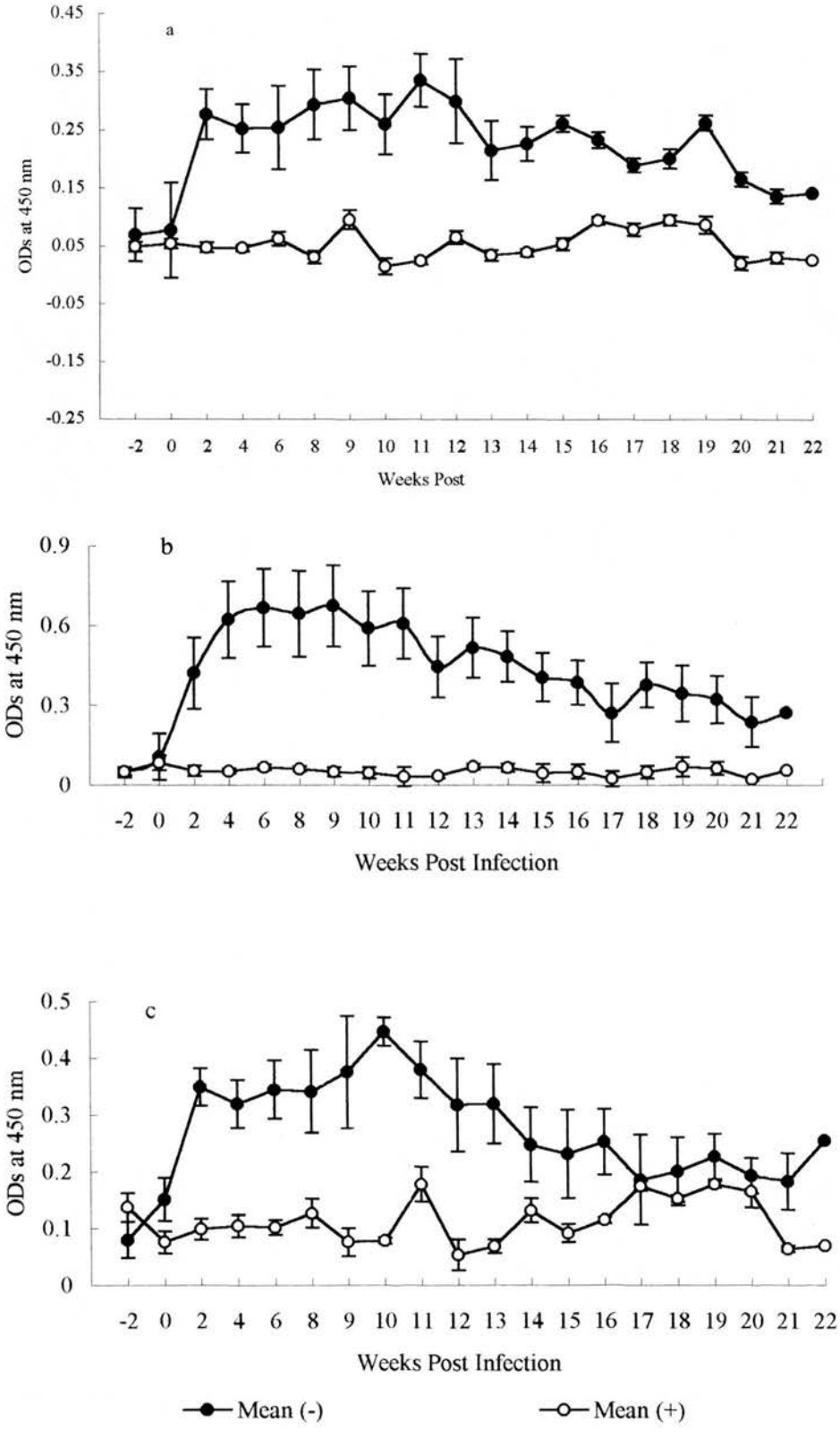


Figure 4.91: The mean ± Sem ELISA OD (450 nm) values for serum total Ig (a), IgG_1 (b) and IgM (c) responses of *F. gigantica* infected sheep (Mean (+)) and uninfected control sheep (Mean (-)) to FhGST

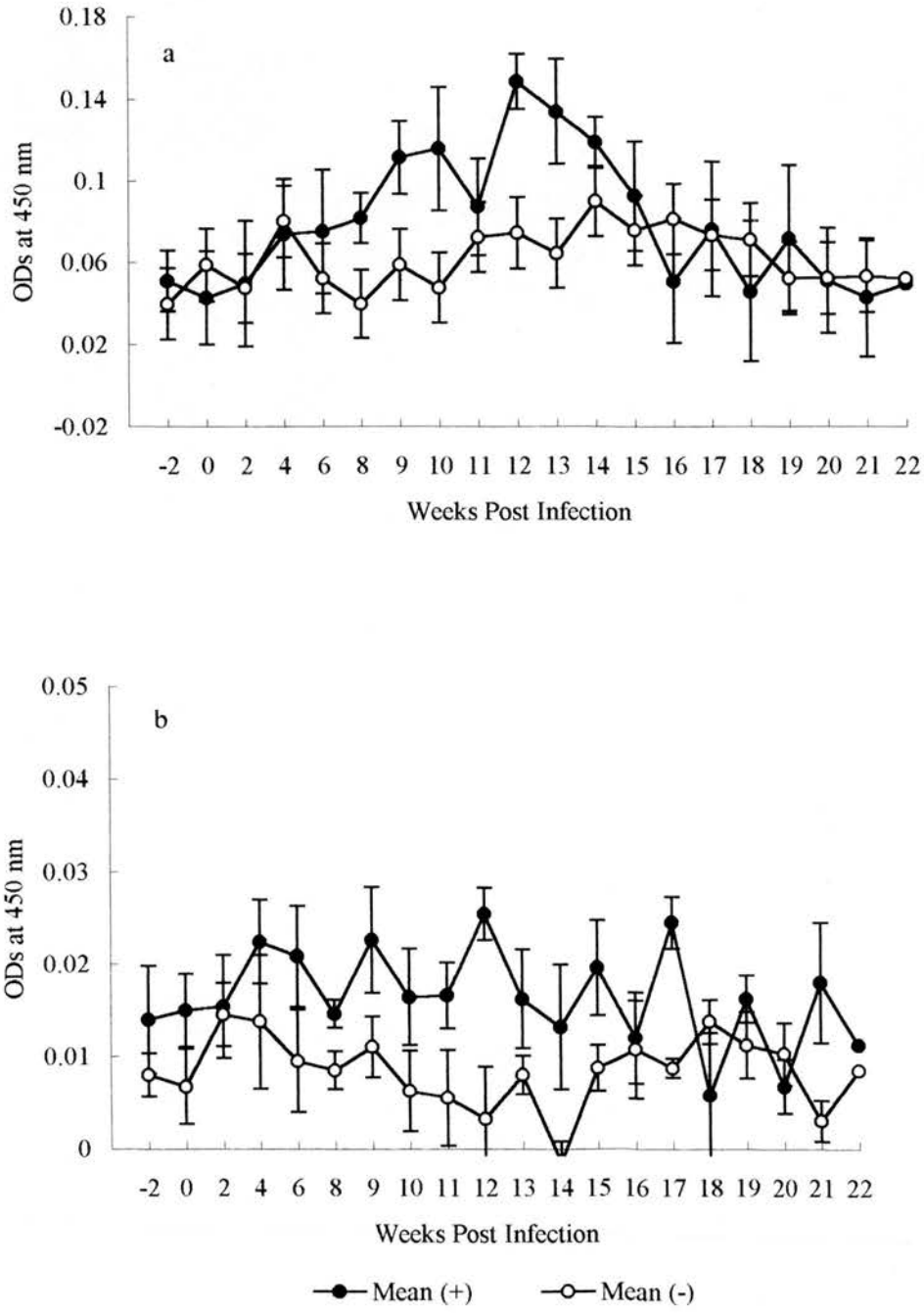


Figure 4.92: The mean ± Sem ELISA OD (450 nm) values for serum IgG_2 (a) and IgM (b) responses of *F. gigantica* infected sheep (Mean (+)) and uninfected control sheep (Mean (-)) to FhGST

4.4.5 Experiment 4: F. *gigantica* (Kenyan Strain) Infection in Sheep

The adjusted mean total Ig ELISA assay results and the isotype-specific antibody responses to Fh-GST of *F. gigantica* infected sheep 23, 25, 27, and 29 and uninfected control sheep 21 and 31 are presented in Figures 4.93a-4.94b and in Appendix tables 4.125-127.

The antibody response to Fh-GST was easily detected in these sera of *F. gigantica* infected animals for total Ig, IgG1 IgM and IgG_2. IgA recorded however was very low.

The infected sheep showed an early (2 wpi.) and sharp total Ig response. The response reached the peak at 2 wpi. for sheep 23 and 25 and 21 wpi. for sheep 27. Sheep 27 also had a late response compared to the rest (Figures 4.93a).

The IgG_1 isotype responses to Fh-GST were stronger in all the *F. gigantica* infected sheep of this group after 2 wpi. The peak IgG_1 responses was 8 wpi. The response slowly and steadily started to reduce but by 21 wpi. however the infected sheep mean OD values were still above the uninfected control sheep by the end of experiment (Figures 4.93b)..

The IgM response to Fh-GST in infected sheep was moderate and late i.e. 6-8 wpi. peaking 10 wpi. before slowly reducing (Figures 4.93c).

There was only one sheep with a clear IgG_2 response to Fh-GST from +8 wpi. as shown in Figure 4.94a

IgA response to Fh-GST was not convincing in this experiment. There was no significant IgA response in serum from these sheep infected with *F. gigantica* (Figure 4.94b).

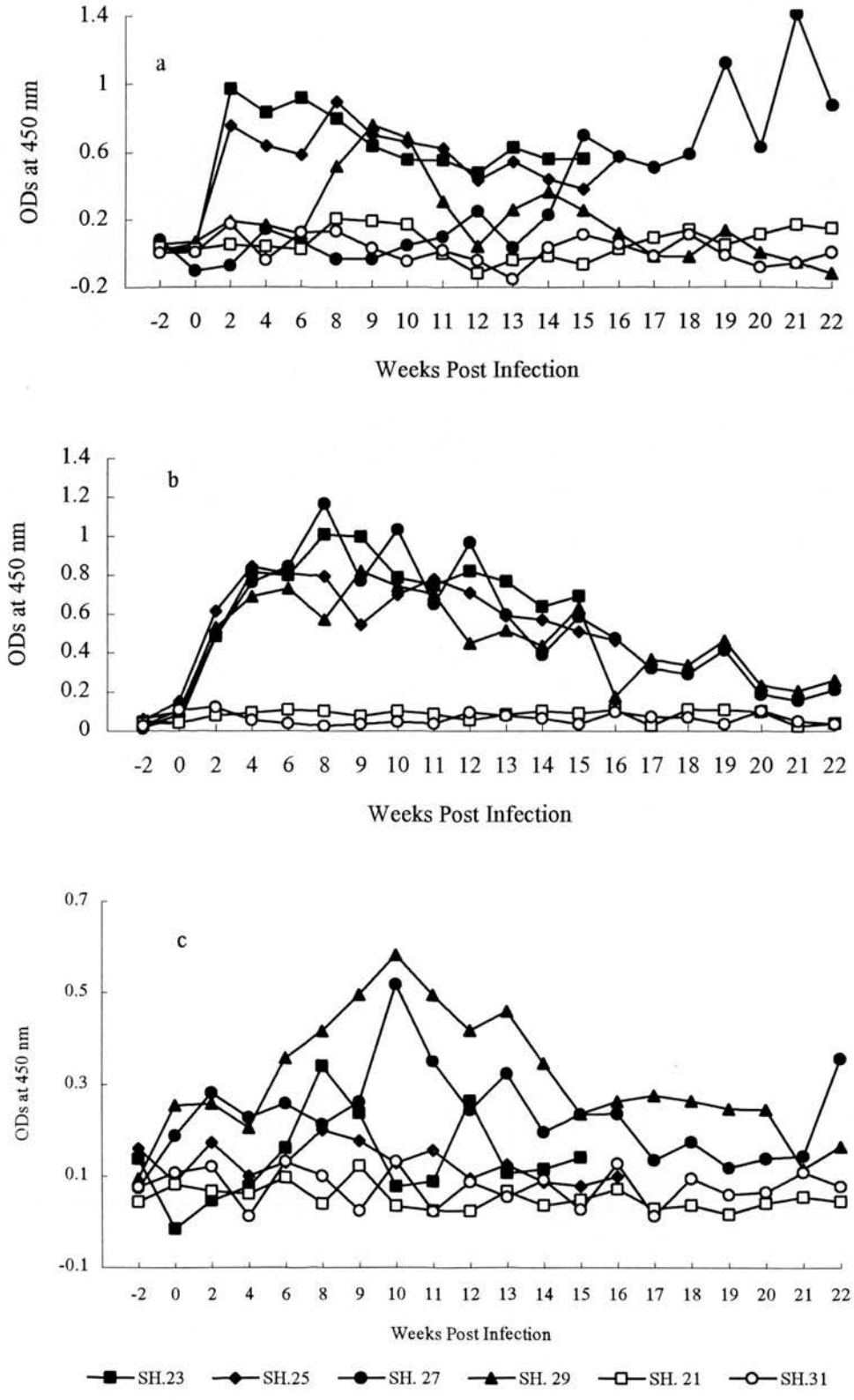


Figure 4.93: The ELISA OD (450 nm) Values for serum total Ig (a), IgG_1 (b) and IgM (c) responses of *F. gigantica* infected sheep (23, 25, 27 and 29) and uninfected control sheep (21 and 31) to (FhGST)

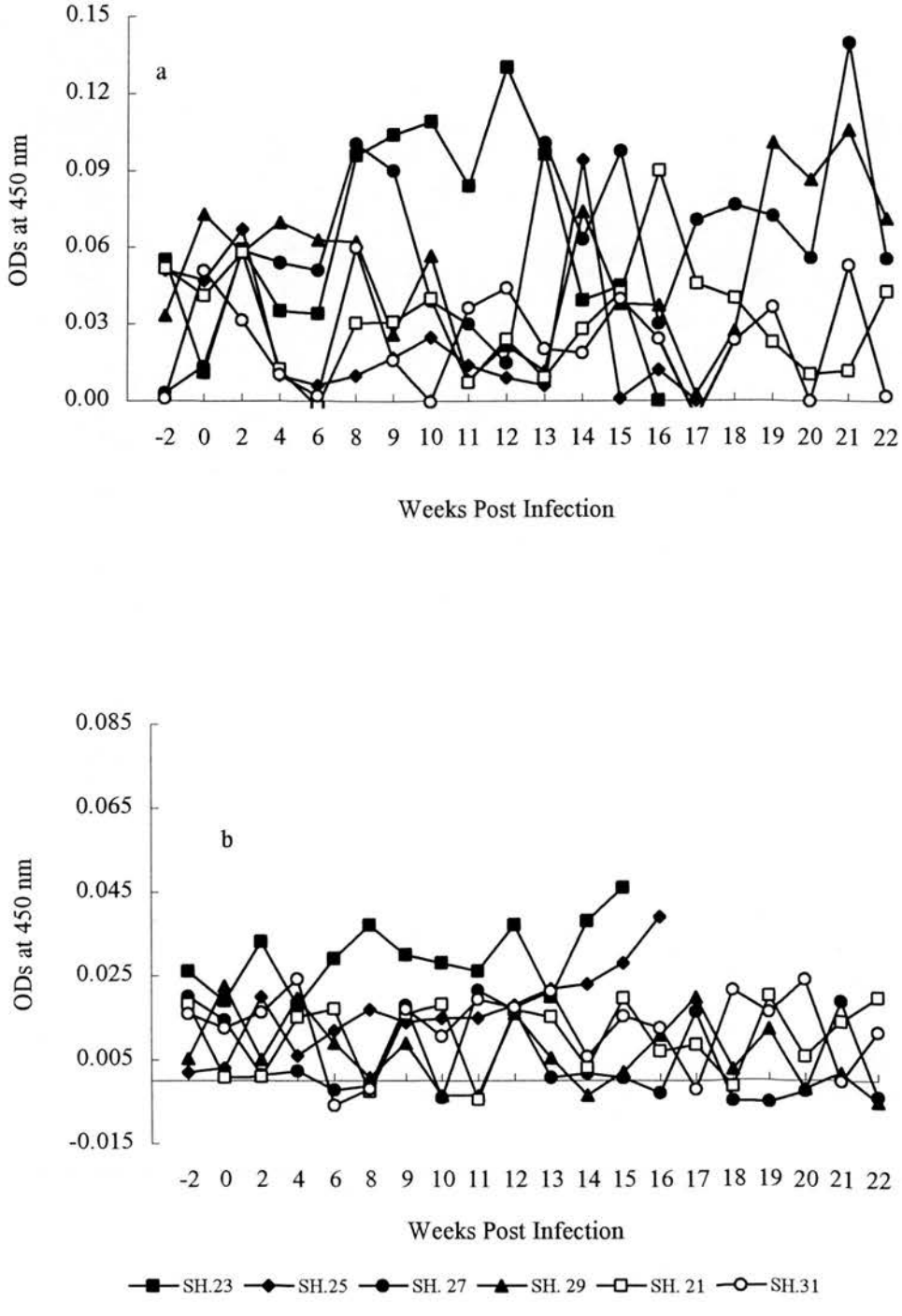


Figure 4.94: The ELISA OD (450 nm) Values for Serum IgG_2 (a) and IgA (b) responses of *F. gigantica* infected sheep (23, 25, 27 and 29) and uninfected control sheep (21 and 31) to Fh-GST

4.4.6 Experiment 5: *F. hepatica* (Peruvian Strain) Infection in Cattle

The ELISA assay was used to detect the total Ig and the isotype-specific antibody responses Fh-GST in *F. hepatica* infected calves. These cattle were either given primary infection as indicated or an infection and a challenge (Calf 15c and 23c) as shown in Table 4.5 in section 4.1.5. The total Ig and the four subclass as are presented in Figures 4.95a-4.96b and the adjusted data in appendix tables 4.128-4.32.

All the infected calves showed a late increase in total Ig levels. The response was on the increase when the experiment was stopped (Figures 4.95a).

IgG_1 isotype responses to Fh-GST was detected in all infected calves of this group. As in total Ig, the IgG_1 response increased slowly. Calves 15c and 23c in response to the circulating IgG_1 antibody showed an increased after the challenge infection. It is clear in this experiment that calves 23c, 34c and 45c with first infection of 600 and 450 metacercariae respectively had a sharper and earlier response than calves 14c and 15c with 200 matacercariae (Figures 4.95b).

Circulating IgM antibody response to Glutathione S-Transferase was much stronger than IgG_1. Calves 15c and 23c showed an increase in response after challenge infection (Figures 4.95c).

In this experiment infected calves serum IgG_2 response was variable. The response was late and calf 23c infected with 600 metacercariae another increase after challenge infection. There was no difference between challenged, calf 23c and unchallenged, calf 14c (Figures 4.96a).

There was a very late IgA response to Fh-GST noticed in calves 14c, 15c and 23c monitored longer enough (Figure 4.96b).

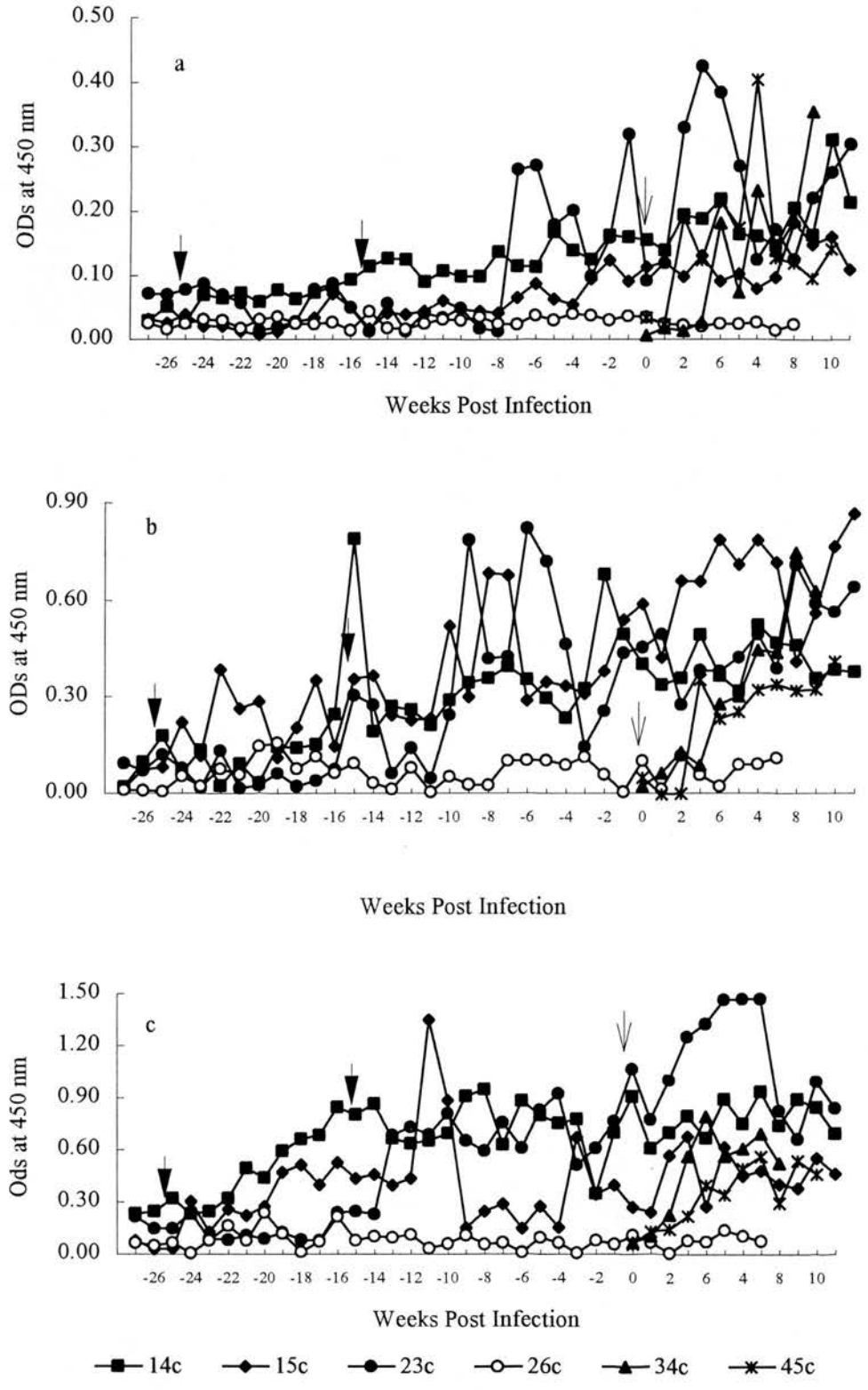


Figure 4.95: The ELISA OD (450 nm) values for total Ig (a), IgG_1 (b) and IgM (c) responses of *F. hepatica* infected calves (14c, 15c, 23c, 34c and 45) and uninfected control calf (26c) to FhGST

→ Primary infection ⇒ Challenge infection

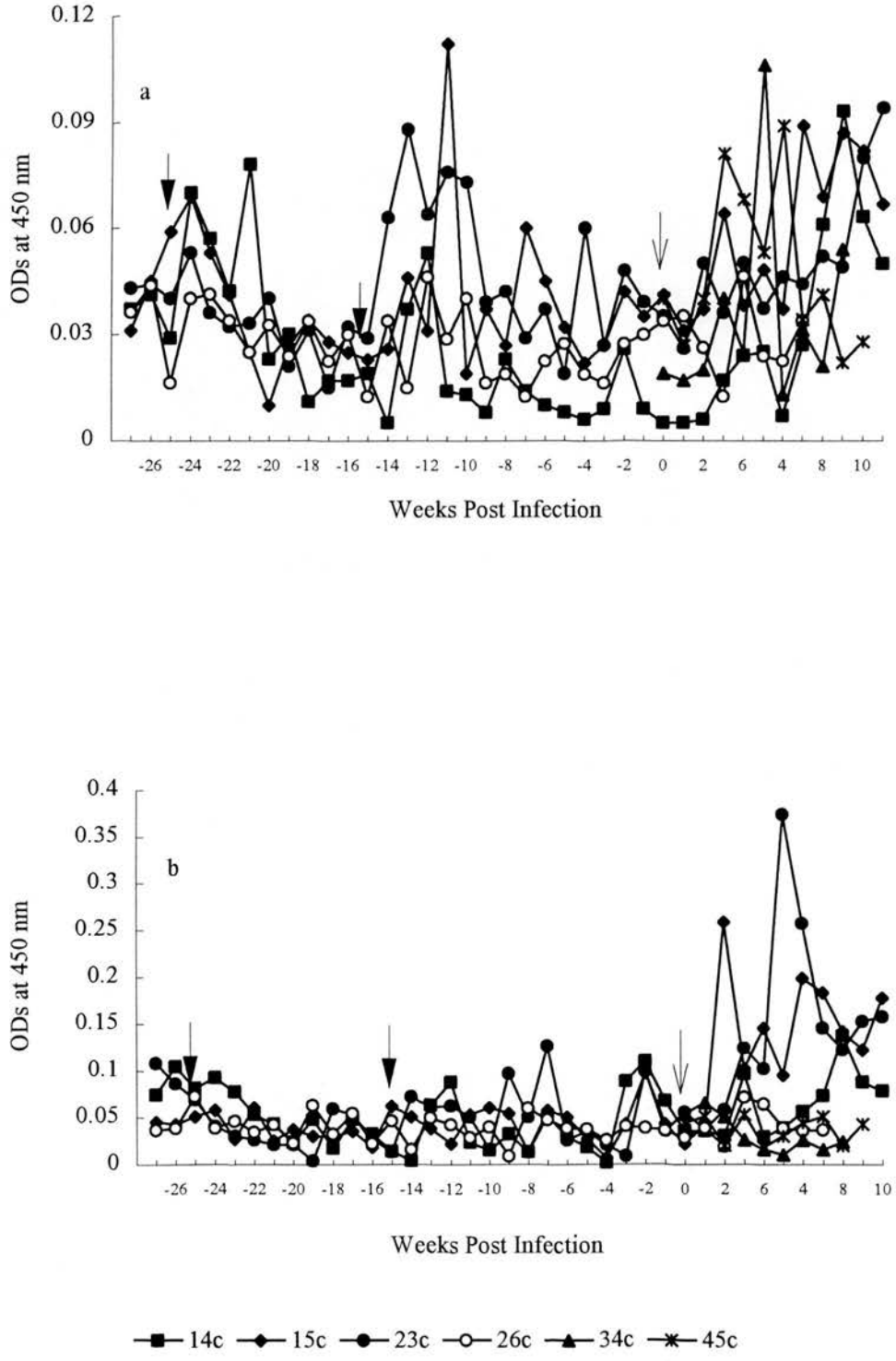


Figure4.96: The ELISA OD (450 nm) values for IgG_2 (a) and IgA (b) responses of *F. hepatica* infected calves (14c, 15c, 23c, 34c and 45c) and uninfected control calf (26c) to FhGST

→ Primary infection ⇒ Challenge infection

4.4.7 Experiment 6: *F. gigantica* (Kenya Strain) Infection in Cattle

The Polyclonal ELISA assay system was used to detect the total Ig and the isotype-specific antibody responses Fh-GST of *F. gigantica* infected calves. Following infection, the Fh-GST antibodies were easily detected in the sera of infected calves for both total Ig and the four subclasses, IgG_1, IgM, IgG_2 and IgA in varying degrees and time of infection. The adjusted total Ig ELISA assay results and the isotype-specific antibody responses to Fh-GST by *F. gigantica* infected Calves 22, 23 and 24 and uninfected control calf 26 are presented in Figures 4.97a-4.98b and adjusted data in appendix tables 4.128-4.32.

All the infected calves showed an increase in total Ig levels from 3 wpi. peaking 3 and 21 wpi. with all calves remaining high throughout the experiment (Figures 4.97a).

Serum IgG_1 responses to Fh-GST was delayed and strong in all the *F. gigantica* infected calves. The values remained high throughout the experiment (Figures 4.97b).

There was a sharp and early (2 wpi.) IgM response to Fh-GST by *F. gigantica* infected calves 22 and 24 while calf recorded a late (11 wpi.) antibody response. The OD values remained high throughout the experiment (Figures 4.97c).

Circulating IgG_2 antibody response to Fh-GST was very poor in calves 22 and 24 while calf 23 response was clear (Figure 4.98a).

A gradual and late IgA response to Fh-GST by *F. gigantica* infected calves was most prominent by calf 22 while the rest remained low (Figure 4.98b).

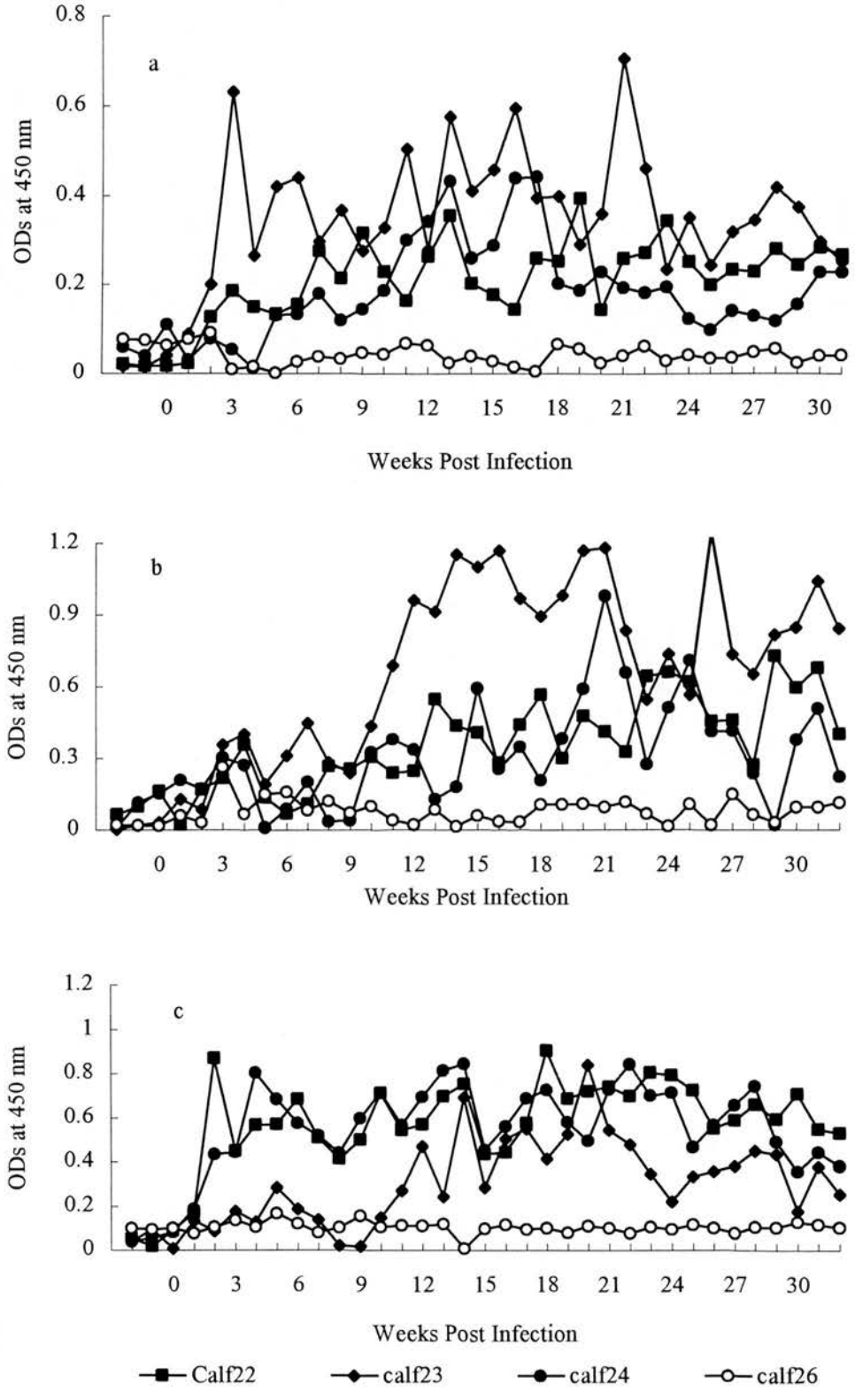


Figure 4.97: The ELISA OD (450 nm) values for total Ig (a), IgG_1 (b) and IgM (c) responses of *F. gigantica* infected calves(22, 23 and 24) and uninfected control calf (26) to Fh-GST

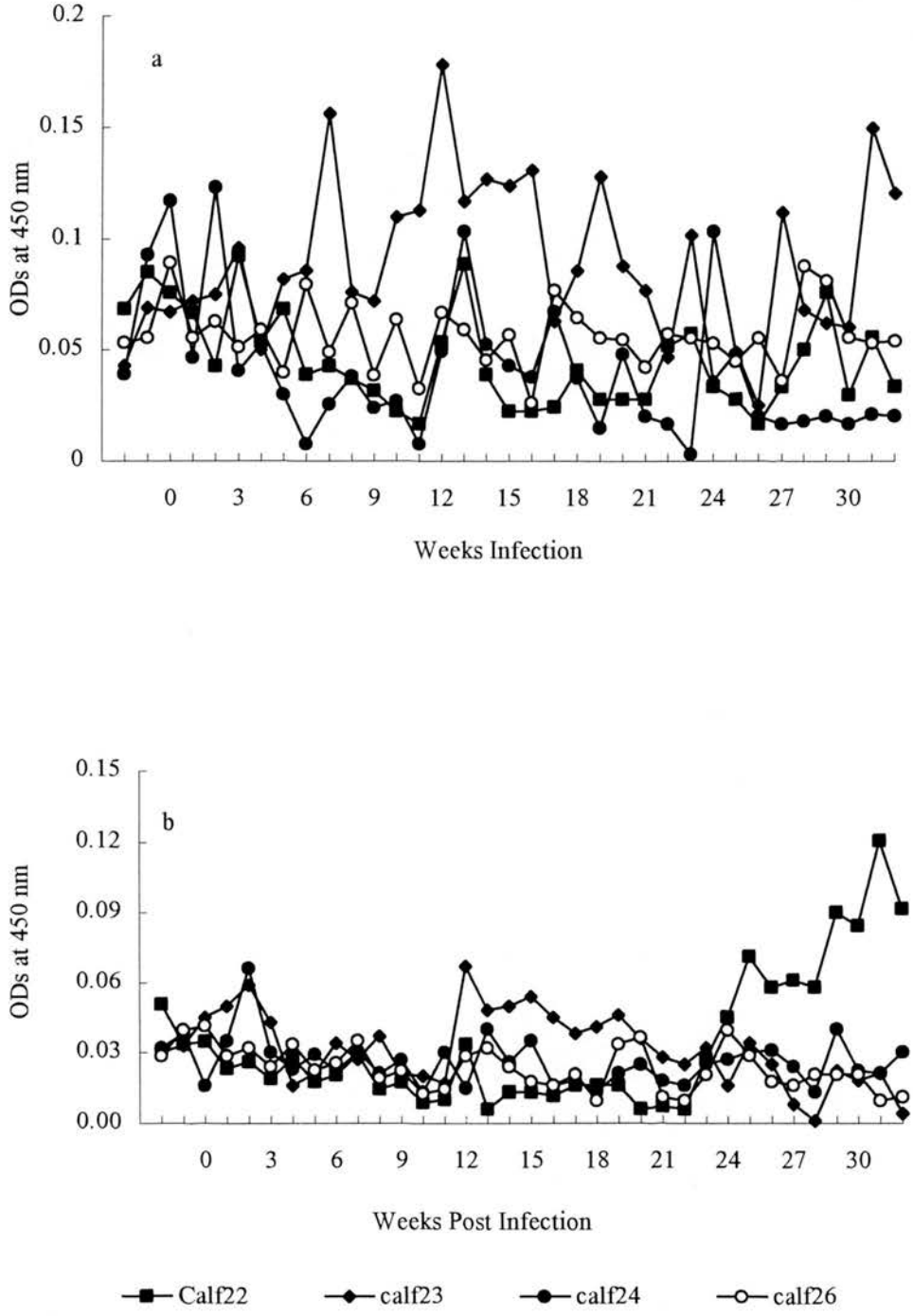


Figure 4.98: The ELISA OD (450 nm) values for IgG_2 (a) and IgA (b) responses of *F. hepatica* infected calves (22, 23 and 24) and uninfected control calf (26) to Fh-GST

4.5 FAECAL ANTIBODY RESPONSES TO *F. HEPATICA* OR *F. GIGANTICA* EXCRETORY/SECRETORY PRODUCTS (FH-E/S OR FG-E/S)

4.5.1 Determination of Optimum Assay Condition by Titration

The optimum assay conditions i.e. those which optimised the signal to the background ratio, were determined by titration of antigen (Fh-E/S or Fg-E/S) and conjugate for the polyclonal detection system or antigen (Fh-E/S or Fg-E/S), monoclonal antibody (McAb) and conjugate for the monoclonal antibody based detection system. Figures 4.99-4.102 are representative titrations for total Ig and IgA in *F. hepatica* and *F. gigantica* infected sheep and cattle for the two detection systems, polyclonal antibody (Ig) and monoclonal antibody (IgA) detection systems (Table 4.17-4.18). Full data is represented in Appendix Tables 4.133-4.137.

Chequerboard titration for monoclonal antibodies and conjugate were carried out by diluting these systems in blocking buffer using doubling serial dilution ranging from 20-1.25µg/ml for Fh-E/S or Fg-E/S, 1:10-1:40 (monoclonal antibody) and 1:1000-1:4,000 (conjugate). Titration were run in triplicate and the mean values calculated. The chosen antigen concentration, monoclonal antibody and conjugate dilution were used in all subsequent sequential screenings. The faecal samples were not titrated, dilution of 1:1 was chosen for both assays. The two positive faecal samples P1 and P2 were taken from *F. hepatica* or *F. gigantica* infected sheep and corresponded to week 8-10 (P1) and p2 week 17-22 (P2) post infection. These times post infection were chosen to assess responses at the middle and at the end of the experimental period.

Table 4.17 Sheep Infected with *F. hepatica* or *F. gigantica*: optimal dilution of antigen (μg/ml), faecal sample and conjugate for polyclonal antibody system using *F. hepatica* or *F. gigantica* excretory/secretory products (Fh-E/S or Fg-E/S).

Assay	*F. hepatica*			*F. gigantica*		
	Fh-E/S	Faeces	Conj.	Fg-E/S	Faeces	Conj.
Total Ig	2.5	1:1	1:2000	2.5	1:1	1:2000
IgM	5	1:1	1:1000	5	1:1	1:1000

Table 4.18 Sheep Infected with *F. hepatica* or *F. gigantica*: optimal dilution of antigen (μg/ml), faecal sample, monoclonal antibody (McAb) and conjugate for monoclonal antibody system using *F. hepatica* or *F. gigantica* Excretory/secretory products (Fh-E/S or Fg-E/S).

Assay	*F. hepatica*				*F. gigantica*			
	Fh-E/S	Faeces	McAb	Conj.	Fg-E/S	Faeces	McAb	Conj.
IgG_1	5	1:1	40	1000	5	1:1	40	1000
IgG_2	5	1:1	20	1000	5	1:1	20	1000
IgA	5	1:1	20	1000	5	1:1	20	1000

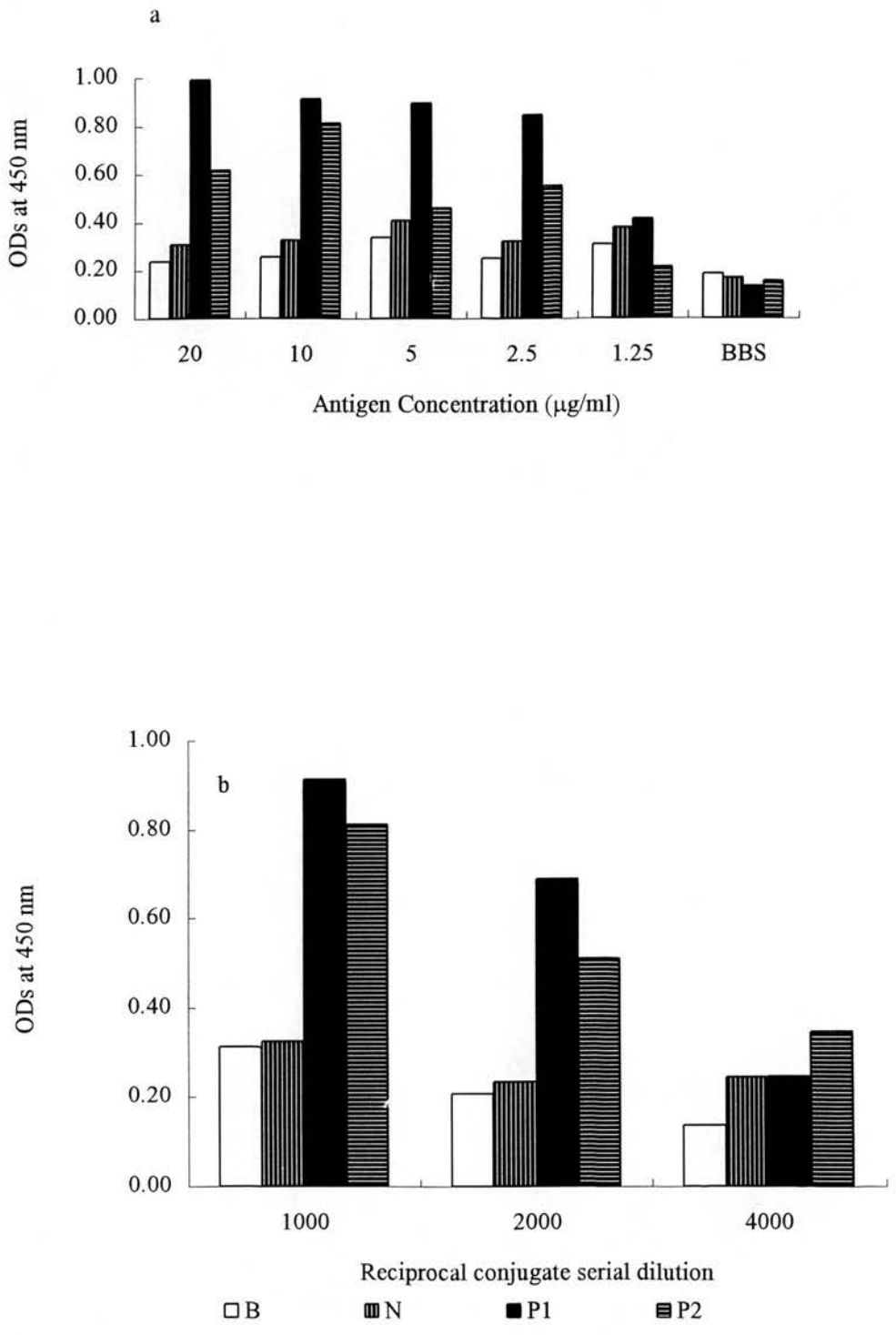
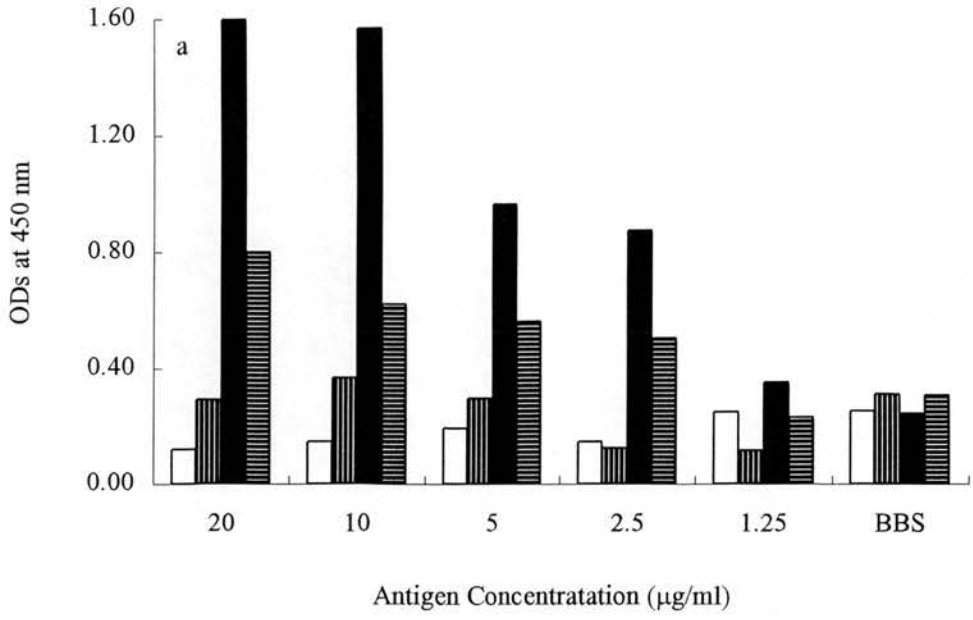


Figure 4.99: Antigen (FgESP) (a) and conjugate (b) titration for total Ig for *F. hepatica* showing the mean ELISA (450 nm) values obtained infected sheep and uninfected control sheep for diluent (B) negative (N) positive (P1) and positive (P2)



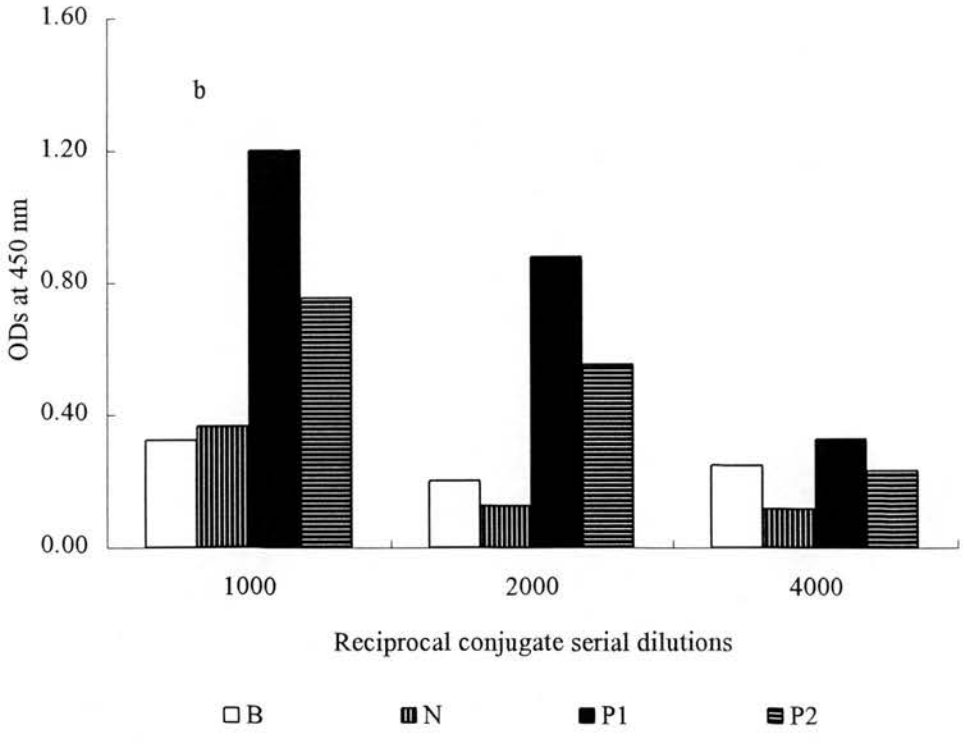


Figure 4.100: Antigen (FgESP) (a) and conjugate (b) titration for total Ig for *F. gigantica* showing the mean ELISA (450 nm) values obtained from infected sheep and uninfected control sheep for diluent (B) negative (N) positive (P1) and positive (P2)

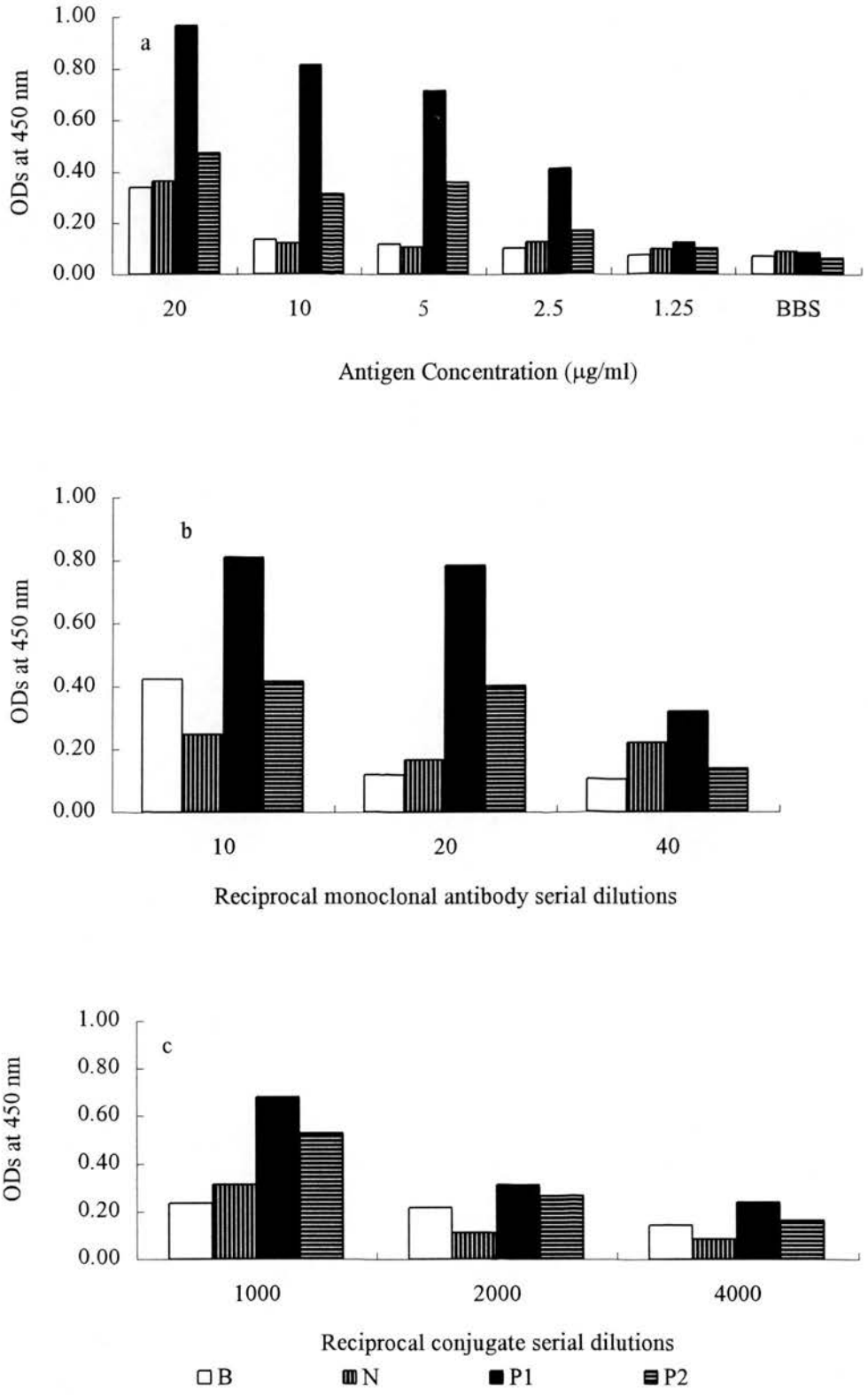


Figure 4.101: Antigen (FhESP) (a), monoclonal antibody (b) and conjugate (c) titration for faecal IgA for *F. hepatica* infected and uninfected control sheep showing the mean ELISA values obtained for diluent (B) negative (N) positive (P1) and positive (P2)

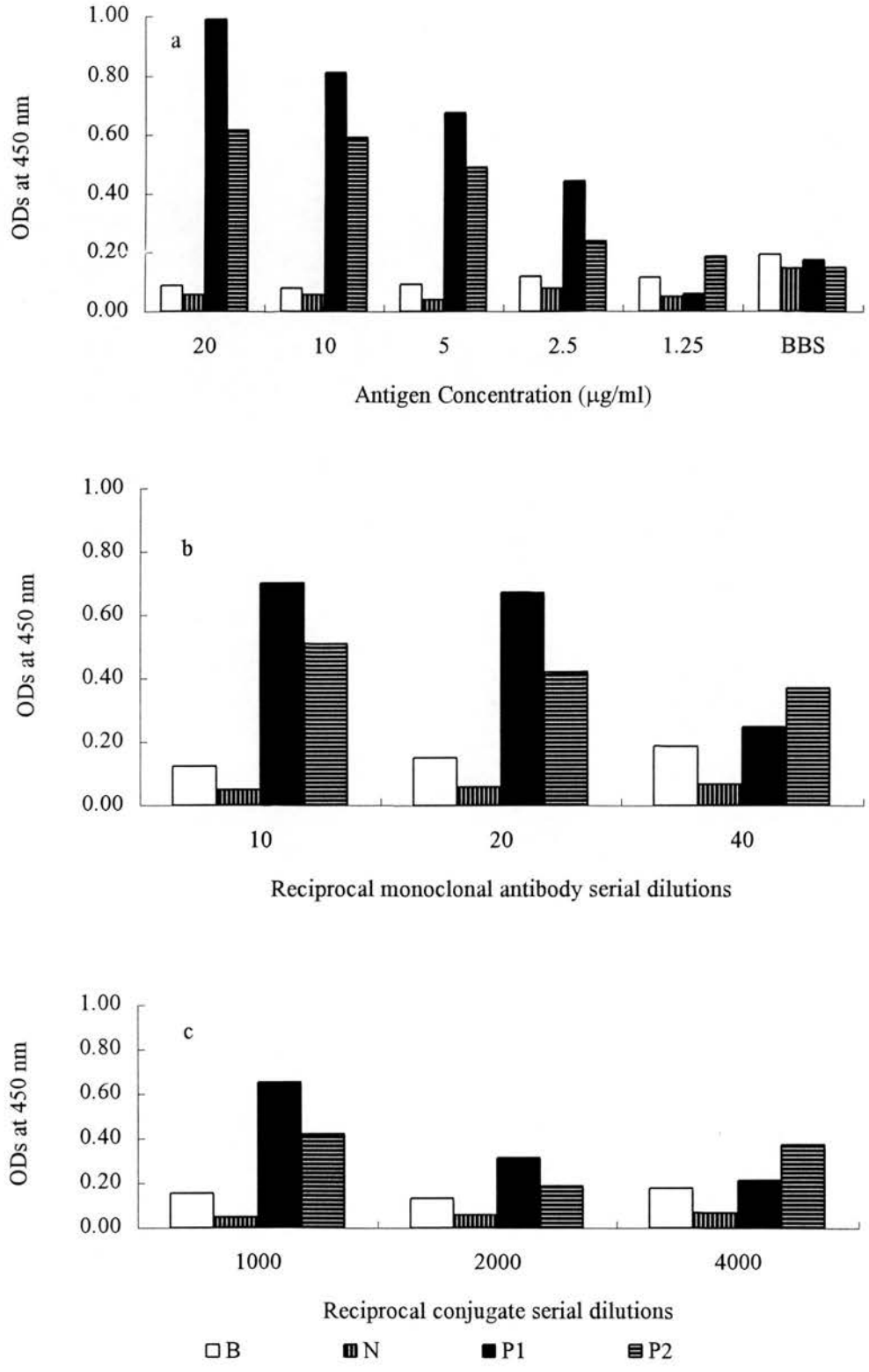


Figure 4.102: Antigen (FhESP) (A), monoclonal antibody (b) and conjugate (c) titrations for faecal IgA for *F. gigantica* infected sheep and uninfected control sheep showing the mean ELISA (450) values obtained for diluent (B) negative (N) positive (P1) and positive (P2)

4.5.2 Experiment 1: *F. hepatica* (Peru and British Strain) Infection in Sheep

Following primary infection, total faecal Ig, IgG_2 and IgA antibodies responses to Fh-E/S were easily detected in the faeces of infected sheep. However there was a slight faecal IgG_2 response to Fh-E/S particularly in sheep 5 and 6 (Figures 4.104a) and faecal IgG_1, and IgM response were not detected (Figures 4.103b and 4.103c). Full data is represented in Appendix tables 4.138-4.140.

All the infected sheep showed an early increase in total Ig levels from 1-4 wpi. peaking 6-8 wpi. and OD values started to reduce by 8-9 wpi. There was very big variation in response within the group with samples from sheep 5 recording the highest values and from sheep 10 the lowest (Figures 4.103a).

F. hepatica infected sheep 5 and 6 faecal IgG_2 levels were high in the earlier part of infection, 2 wpi. The rest of the sheep recorded the same OD values as the uninfected sheep (Figures 4.104a).

Faecal IgA response to Fh-E/S was the dominant isotype with biphasic response in sheep 6 and 9, the first peak was at 2-3 wpi., before patency and the second peak was after patency at 15 wpi. (Figures 4.104b).

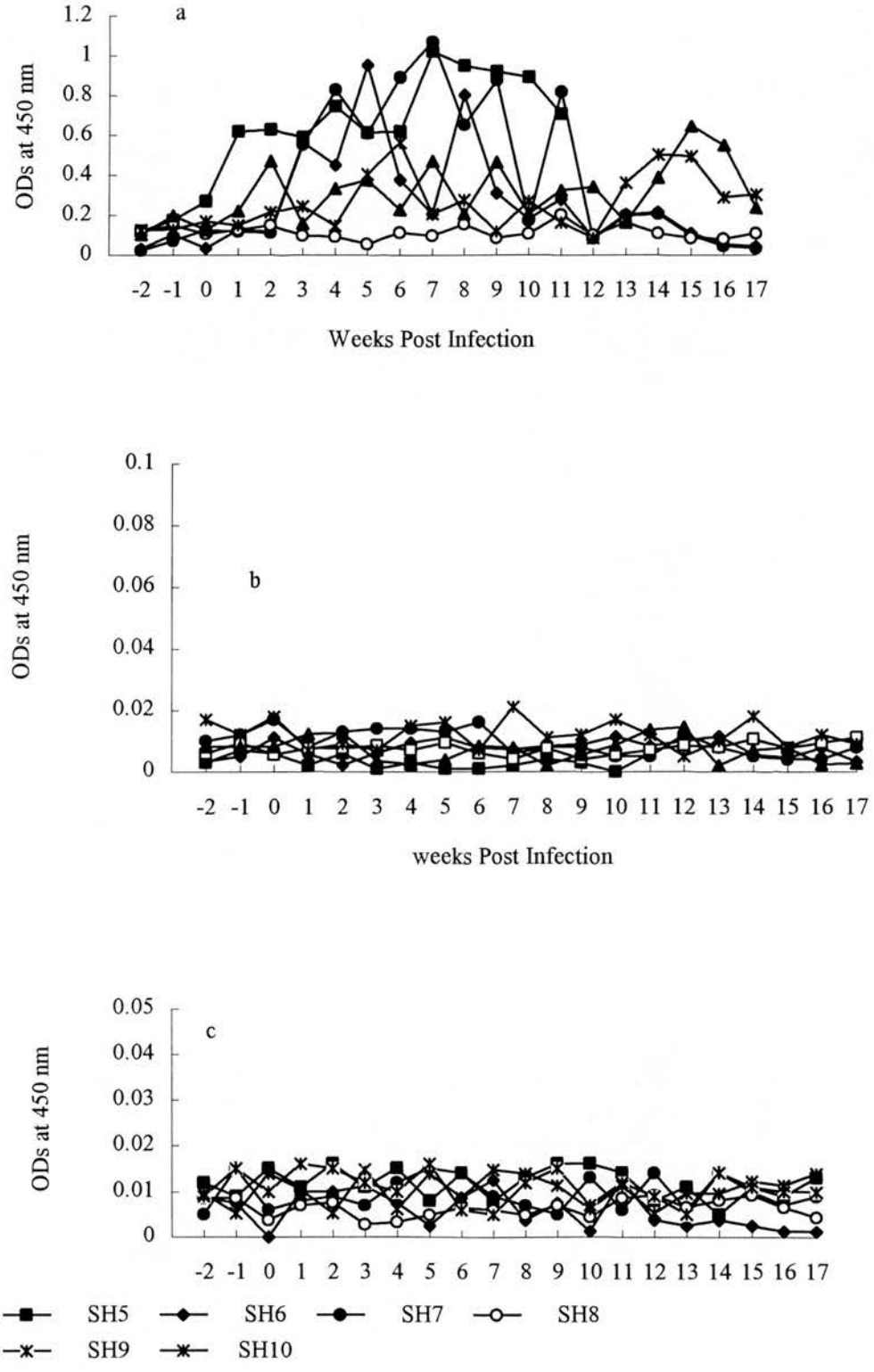


Figure 4.103: The adjusted ELISA OD (450 nm) faecal total Ig (a), IgG_1 (b) IgM (c) responses of *F. hepatica* infected sheep 5, 6, 7, 9 and 10 and uninfect control sheep 6 to *F. hepatica* excretory and secretory products (FhESP)

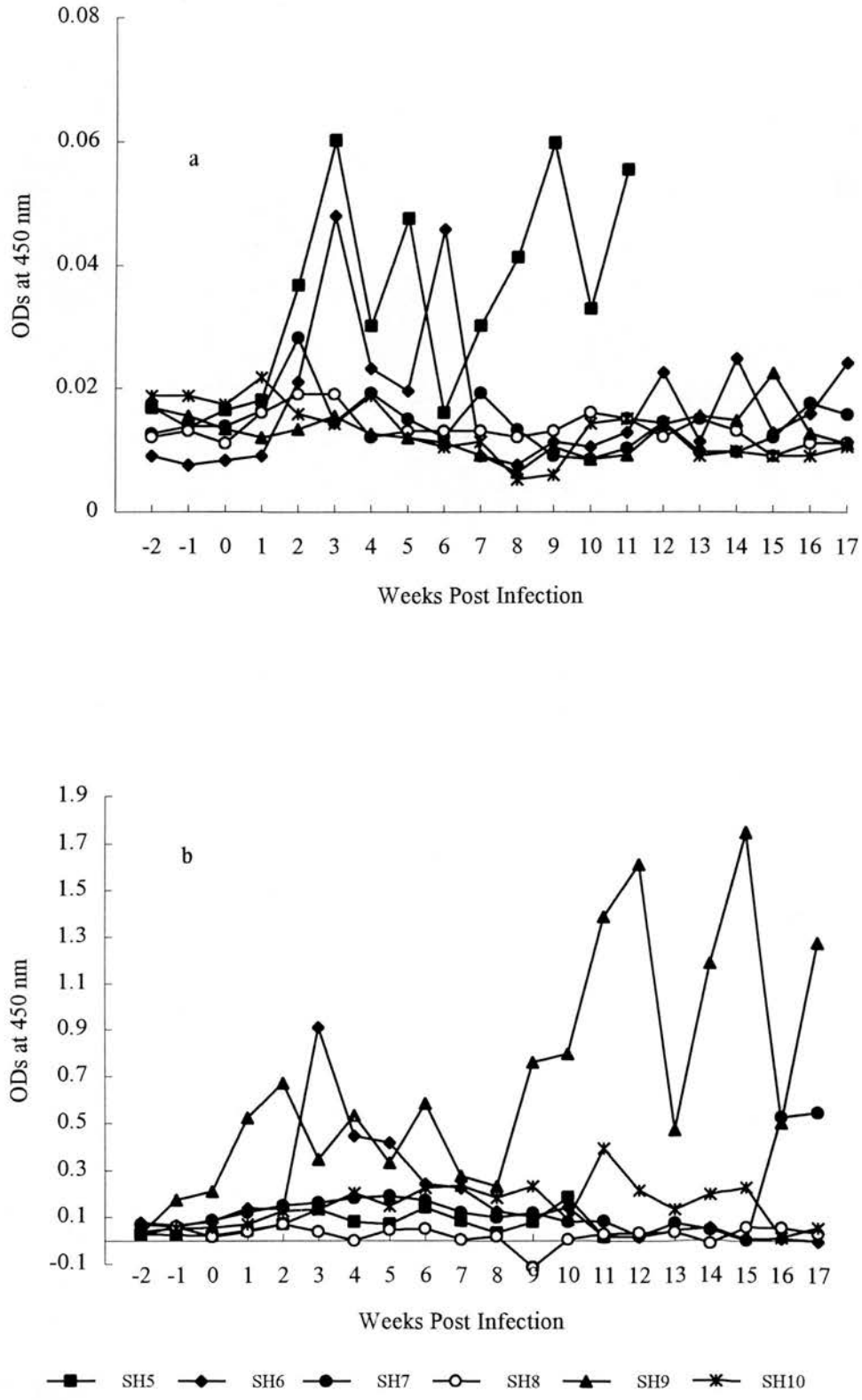


Figure 4.104: The adjusted ELISA ODs (450 nm) faecal IgG_2 (a) and IgA (a) responses of *F. hepatica* infected sheep 5, 6, 7 and 9 and 10 uninfected control sheep 8 to *F. hepatica* excretory and secretory products (Fh-E/S)

4.5.3 Experiment 3: *F. gigantica* (Kenyan Strain) Infection in Sheep

Total faecal Ig and IgA response to Fg-E/S was detected in *F. gigantica* infected sheep but the IgM, IgG_1 and IgG_2 responses were low. The mean(±SEM) antibody responses to Fg-E/S of *F. gigantica* infected sheep and uninfected control sheep are presented in Figures 4.105-4.106.

The infected sheep showed an early increase in total Ig levels, 4 wpi. and peaking at 14 wpi. The mean OD levels started slowly to drop from 15 wpi. By the end of experiment however the mean OD values in infected sheep were significantly higher ($p < 0.01$) than the uninfected controls.

In this experimentally *F. gigantica* infected sheep, there was poor faecal IgG_2 response to Fg-E/S.

Faecal IgA response to Fg-E/S *F. gigantica* was biphasic with the first peak 4-8 wpi. followed by a sharp drop 9 wpi. and a second peak 19-21 wpi. The adjusted data in Appendix tables 4.141-4.145

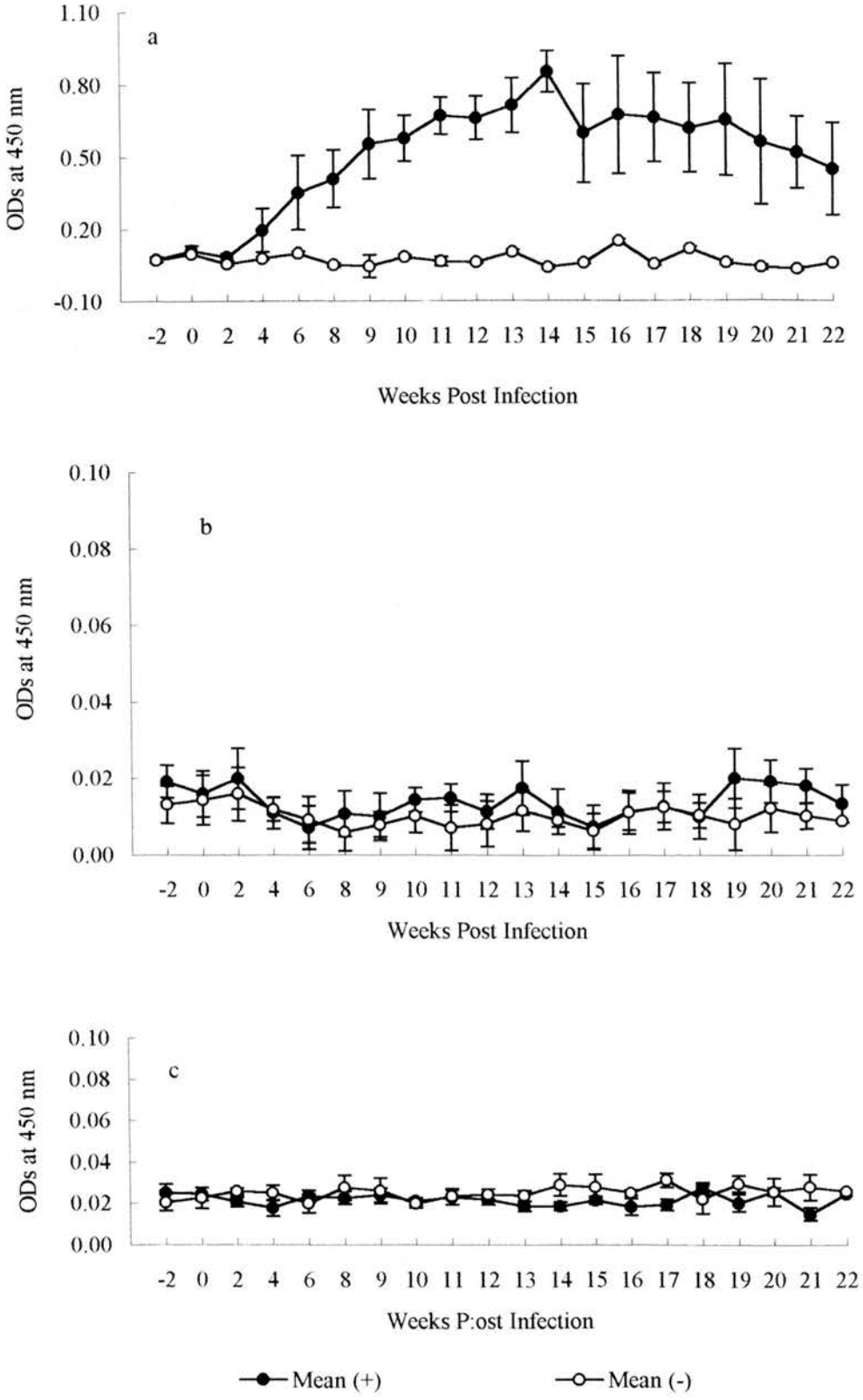


Figure 4.105: The adjusted ELISA OD (450 nm) mean faecal total Ig (a), IgG (b) and IgM (c) responses of *F. gigantica* infected sheep (+) and uninfected control sheep (-) to *F. gigantica* and secretory products (Fh-E/S)

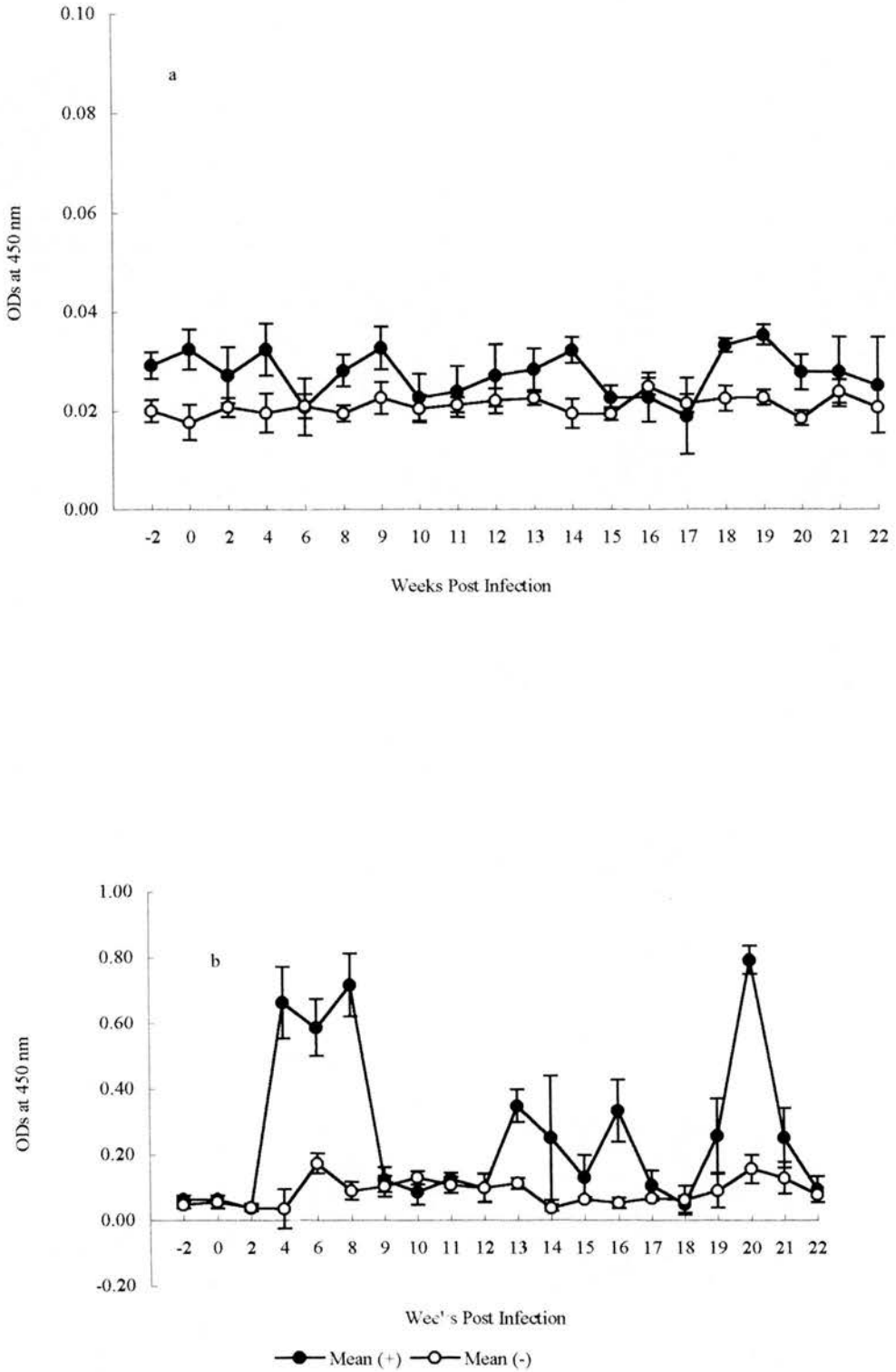


Figure 4.106: The adjusted ELISA OD (450 nm) mean faecal IgG_2 (a) and IgA (b) responses of *F. gigantica* infected sheep (+) and uninfected control sheep (-) to *F. gigantica* and secretory products (Fh-E/S)

4.6 FAECAL ANTIBODY RESPONSES TO FH-CATHEPSIN.

4.6.1 Determination of Optimum Assay Conditions by Titration.

The optimum assay conditions i.e. those which optimised the signal to the background ratio, were determined by titration of antigen (Fh-Cathepsin) and conjugate for the polyclonal detection system or antigen (Fh-Cathepsin), monoclonal antibody and conjugate for the monoclonal antibody based detection system. Figures 4.107-4.110 are representative titrations for total Ig and IgA in *F. hepatica* and *F. gigantica* infected sheep and cattle for the two detection systems, polyclonal antibody (Ig) and monoclonal antibody (IgA) detection systems. The selected concentrations for the assays IgM, IgG2 and IgA for sheep are in Table 4.19. and 4.20. Full data is represented in Appendix table 4.146-4.150.

Chequerboard titration for monoclonal antibodies and conjugate were carried out by diluting these systems in blocking buffer using doubling serial dilution ranging from 4-1µg/ml for antigen, 1:10-1:40 for monoclonal antibody and 1:1000-1:4000 for conjugate. Titrations were run in triplicate and the mean values calculated. The chosen antigen concentration, faecal samples (1:1), monoclonal antibody and conjugate dilution were used in all subsequent sequential screenings. Dilutions were selected on the basis of optimising the signal to background ratio except in faecal samples where a 1:1 dilution was selected as the highest dilution without titration. The two positive faecal samples P1 and P2 were taken from *F. hepatica* or *F. gigantica* infected sheep and corresponded to week 8-10 (P1) and week 21-22 (P2) post infection. These times post infection were chosen to assess responses at the

middle and at the end of the experimental period and to optimise the signal to background ratio.

Table 4.19 Sheep infected with *F. hepatica* or *F. gigantica*: optimal dilution of antigen i.e. Fh-Cathepsin (μg/ml), faecal sample and conjugate for polyclonal antibody system using *F. hepatica* Cathepsin L Protease (Fh-Cathepsin)

Assay	*F. hepatica*			*F. gigantica*		
	Fh-Cathepsin	Faeces	Conj.	Fh-Cathepsin	Faeces	Conj.
Total Ig	1	1:1	1:1000	1	1:1	1:1000
IgM	1	1:1	1:1000	1	1:1	1:1000

Table 4.20 Sheep infected with *F. hepatica* or *F. gigantica*: optimal dilution of antigen (μg/ml), Faecal Sample, monoclonal antibody (McAb) and conjugate for monoclonal antibody system using *F. hepatica* Cathepsin L Protease (Fh-Cathepsin)

Assay	*F. hepatica*				*F. gigantica*			
	Fh-Cathepsin	Faeces	McAb	Conj.	Fh-Cathepsin	Faeces	McAb	Conj.
IgG_1	1	1:1	1:20	1:1000	1	1:1	1:20	1:1000
IgG_2	1	1:1	1:20	1:1000	1	1:1	1:20	1:1000
IgA	1	1:1	1:20	1:1000	1	1:1	1:20	1:1000

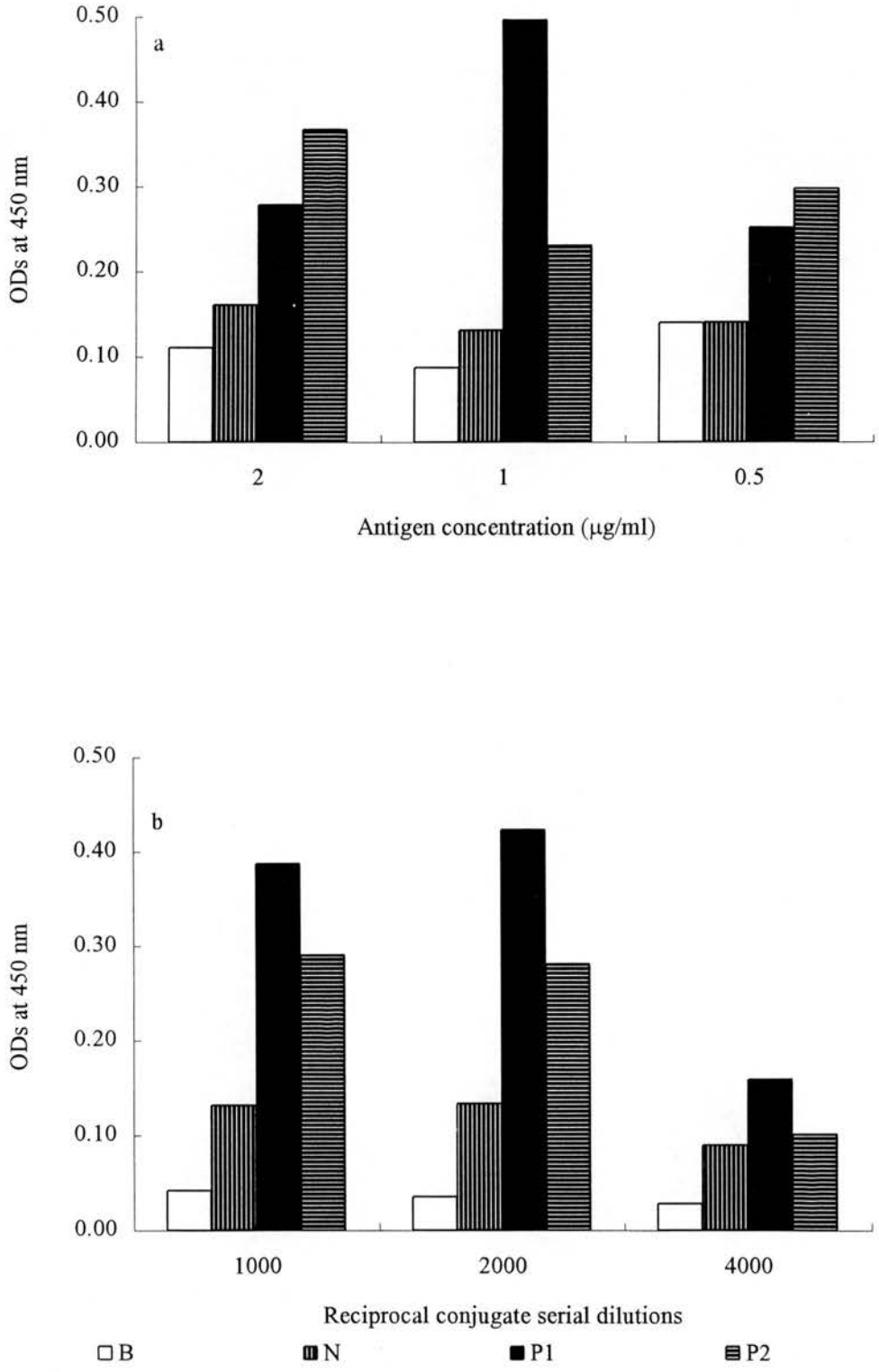


Figure 4.107: Antigen (Fh-cathepsin) (a) and conjugate (b) titrations for total Ig for *F. hepatica* showing the mean ELISA (450 nm) values obtained for infected and uninfected control sheep for diluent (B), negative (N), positive (P1) and positive (P2)

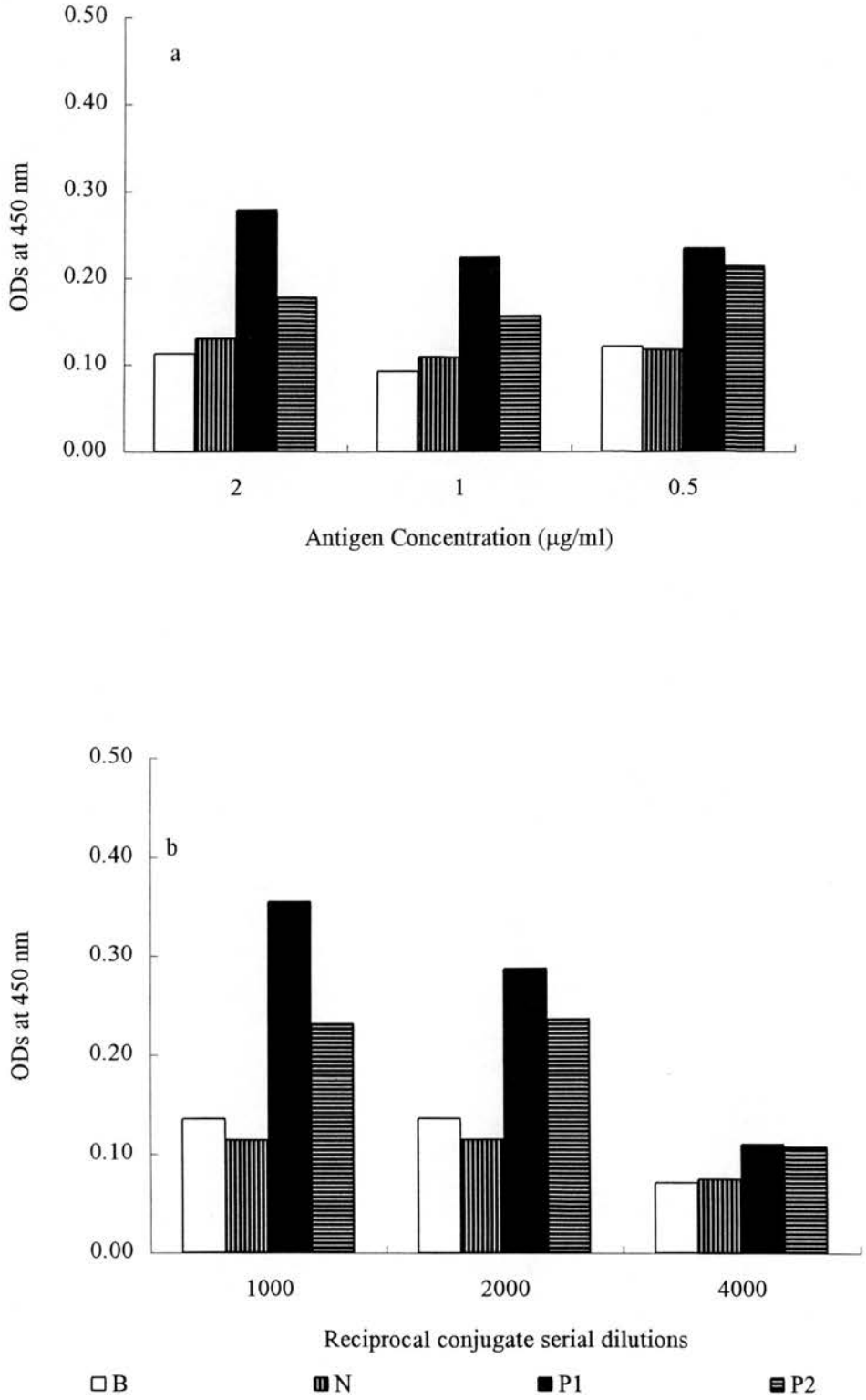


Figure 4.108: Antigen (Fh-cathepsin) (a) and conjugate (b) titrations for total Ig for *F. hepatica* showing the mean ELISA (450) values obtained for infected and uninfected sheep for diluent (B), negative (N), positive (P1) and positive (P2)

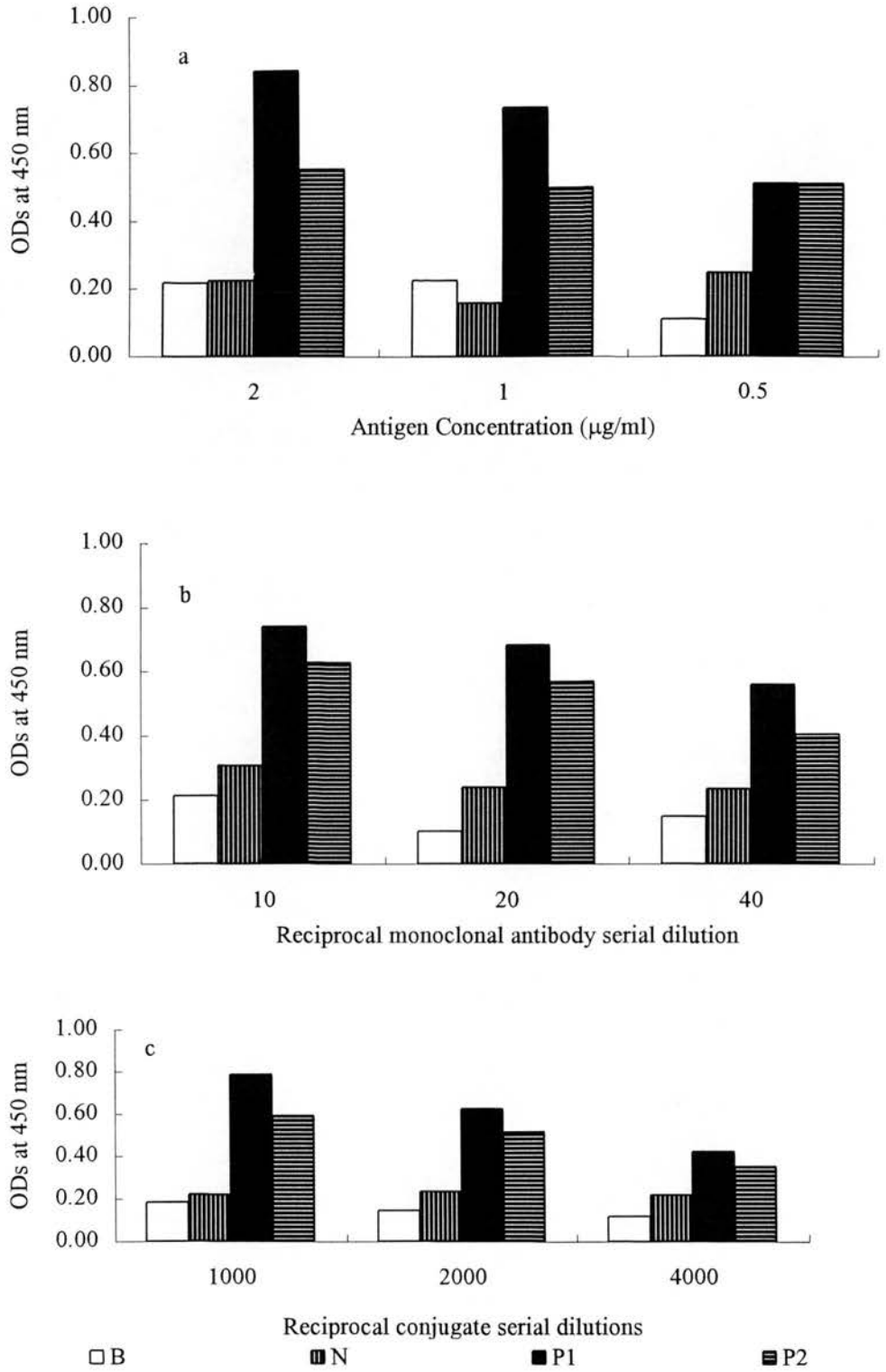


Figure 4.109: Antigen (Fh-Cathepsin) (a), monoclonal antibody (b) and titrations for faecal IgA for *F. hepatica* infected sheep and uninfected sheep conjugate (c) showing mean ELISA (450 nm) values obtained for diluent (B) negative (N) positive (P1) and positive (P2)

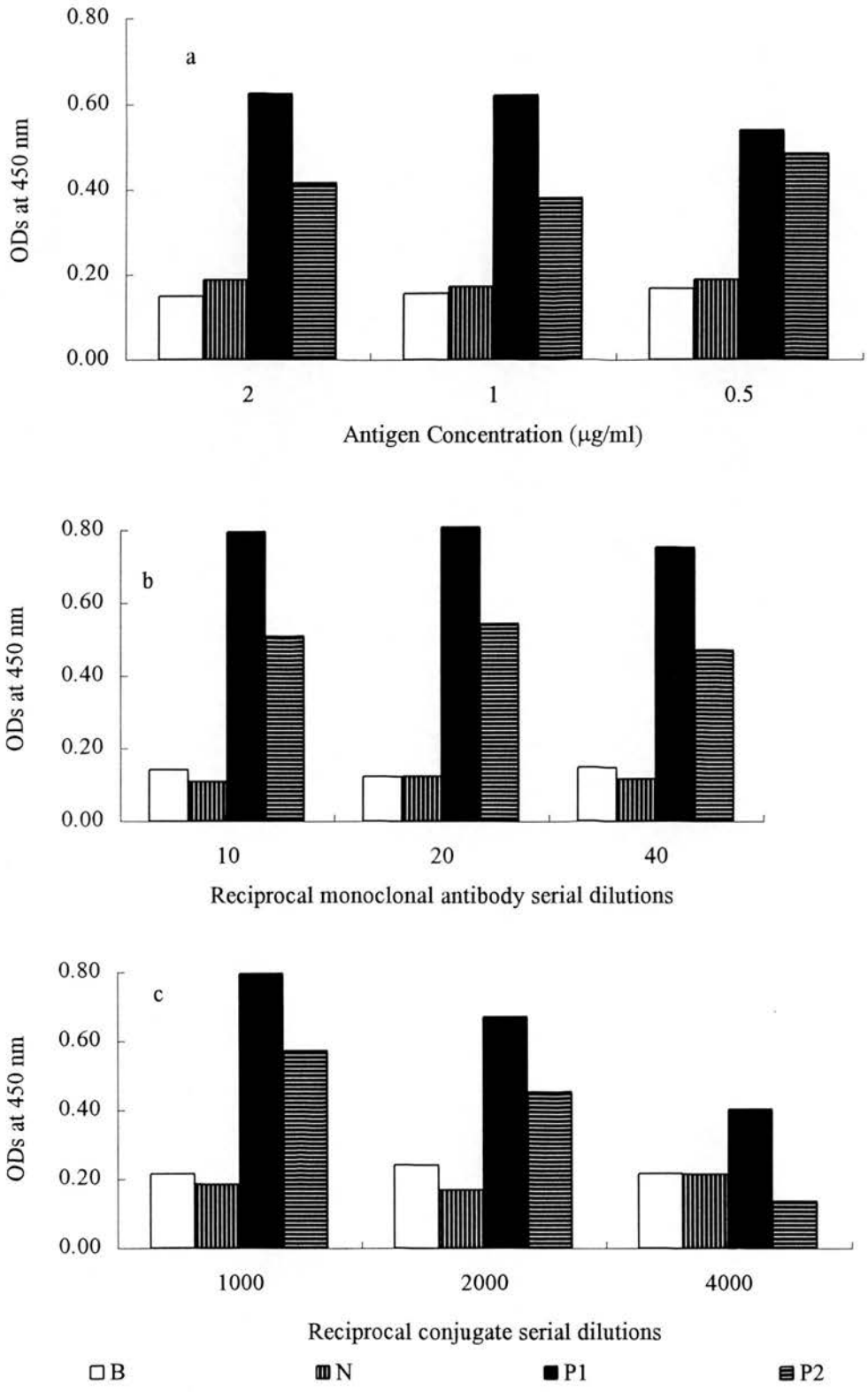


Figure 110: Antigen (Fh-cathepsin) (a), monoclonal antibody (b) and conjugate (c) titrations for feacal IgA for *F. gigantica* infected sheep and uninfected sheep showing mean ELISA (450 nm) values obtained for diluent (B) negative (N) positive (P1) and positive (P2)

4.6.2 Experiment 1: *F. hepatica* (Peruvian And British Strain) Infection in Sheep

Following primary infection, only total faecal Ig and IgA antibody responses to Fh-Cathepsin were detected in the infected sheep. There was a no significant faecal IgG_1, IgM, and IgG_2 response (Figures 4.111-112).

All the infected sheep showed an early increase in total faecal Ig levels from 2 wpi. peaking at 6-7 wpi. OD values then started to decrease by 8 wpi., with a peak again at 12 wpi. There were variations in IgA response within the group.

Faecal IgA response to Fh-Cathepsin was similar to total Ig. Faecal IgA response was clearly biphasic in all the infected sheep . The first phase was started 2 wpi. and started to fall by 8 wpi. and the second and shorter phase started 13 wpi. and started to fall 16 wpi. Full data is represented in Appendix table 4.151-4.153

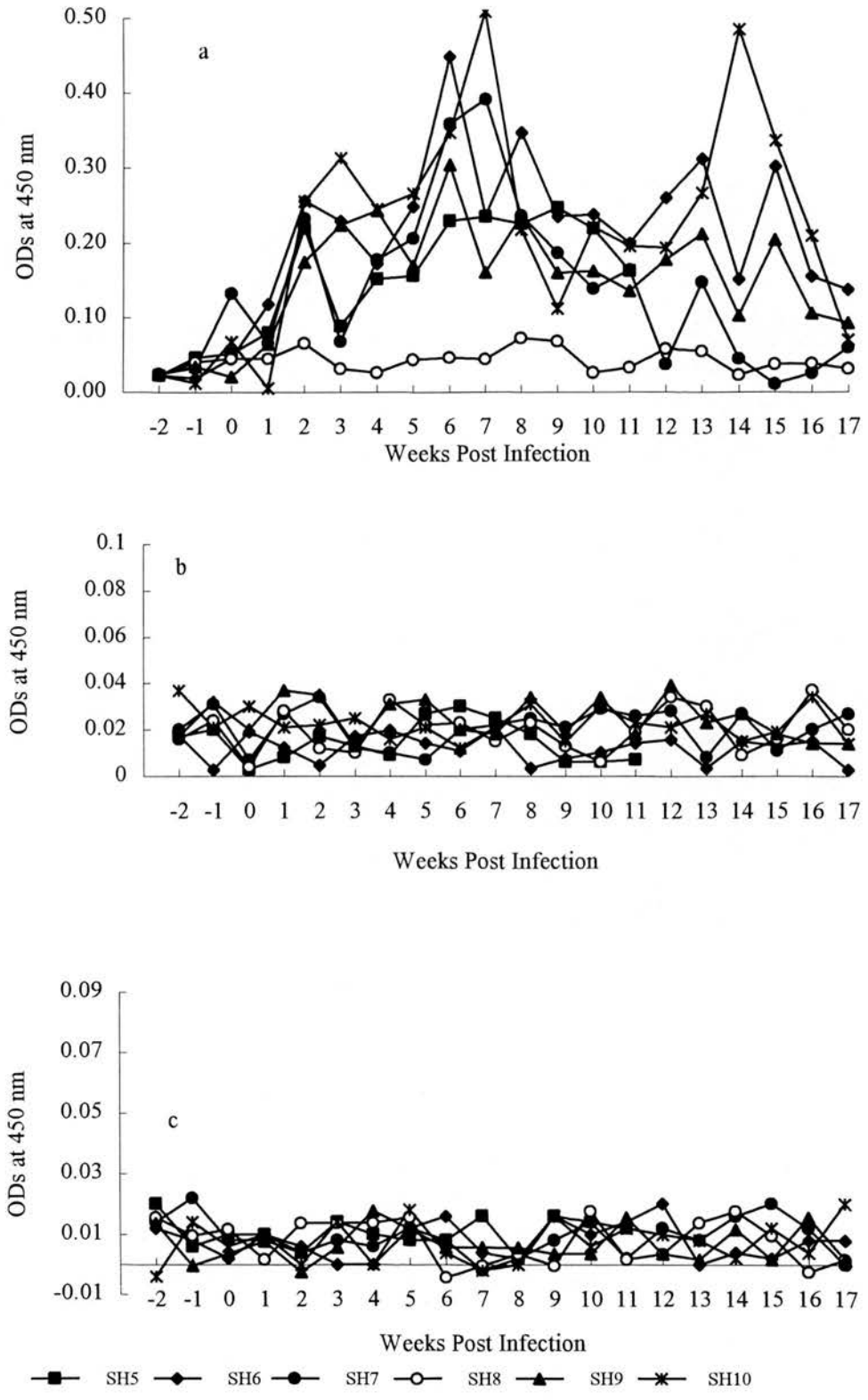


Figure 4.111: The adjusted ELISA OD (450 nm) faecal total Ig (a), IgG_1 (b) and IgM (c) responses of *F. hepatica* infected sheep 5, 6, 7, 9 and 10 and uninfected control sheep 6 to *F. hepatica* Fh-Cathepsin

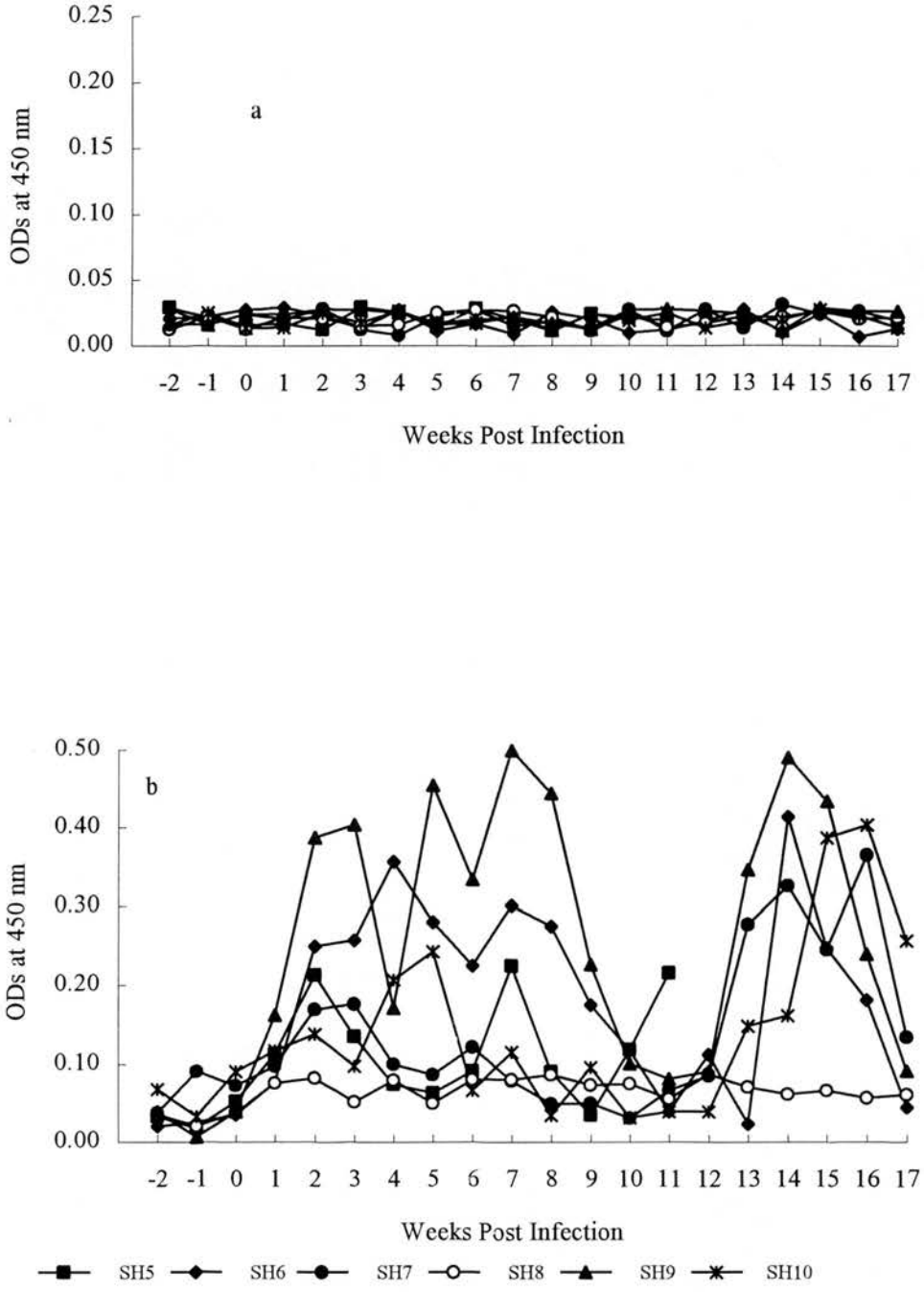


Figure 4.112: The adjusted ELISA OD (450 nm) faecal IgG_2 (a) and IgA (b) responses of F. hepatica infected sheep 5, 6, 7 and 9 and 10 uninfected contro sheep 8 to Fh-cathepsin

4.6.3 Experiment 3: *F. gigantica* (Kenyan Strain) Infection in Sheep

Only total faecal Ig and IgA antibody responses to Fh-Cathepsin were detected in the samples from *F. gigantica* infected animals. IgM, IgG_1 and IgG_2 responses were low to non existence (Figures 4.113-114)..

The infected sheep showed an early increase in total Ig levels, 4 wpi. and peaked at 19 wpi. The mean OD levels started to drop from 22 wpi. By the end of experiment however the mean OD values in infected sheep were almost at the same level as that of uninfected control sub group.

There was an early, 4 wpi., sharp faecal IgA response to Cathepsin L Protease *F. gigantica* infected sheep with two phases, the peak was 10 wpi. for the first phase and 16 wpi. for the second phase. The second phase was more pronounced than the first one followed by a fall 20 wpi. Full data is represented in Appendix table 4.154-4.158.

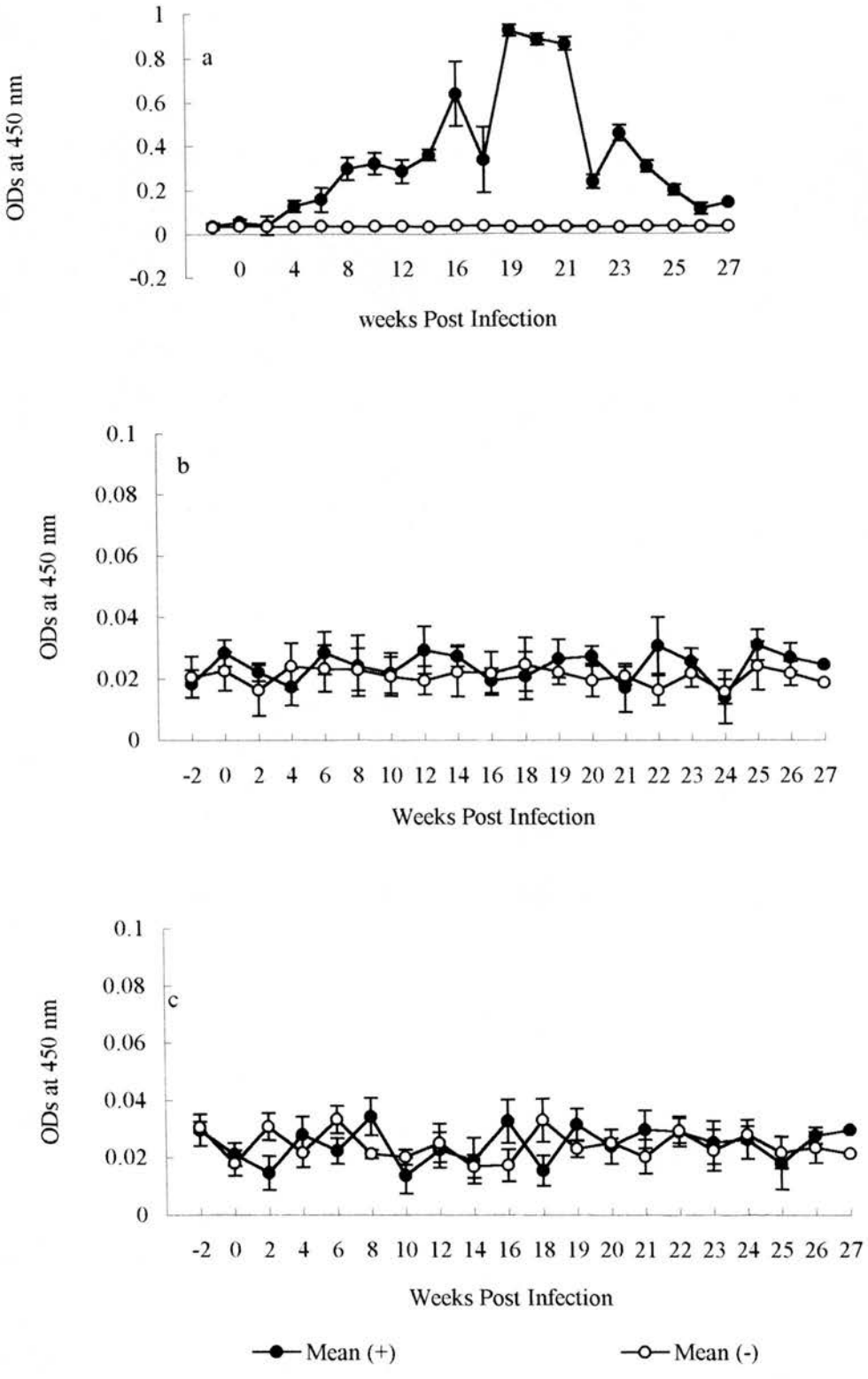


Figure 4.113: The adjusted ELISA OD (450 nm) mean ± SEM faecal total Ig (a), IgG_1 (b) and IgM (c) responses of *F. gigantica* infected sheep 11-15 and uninfected control sheep 16-19 to Fh-Cathepsin

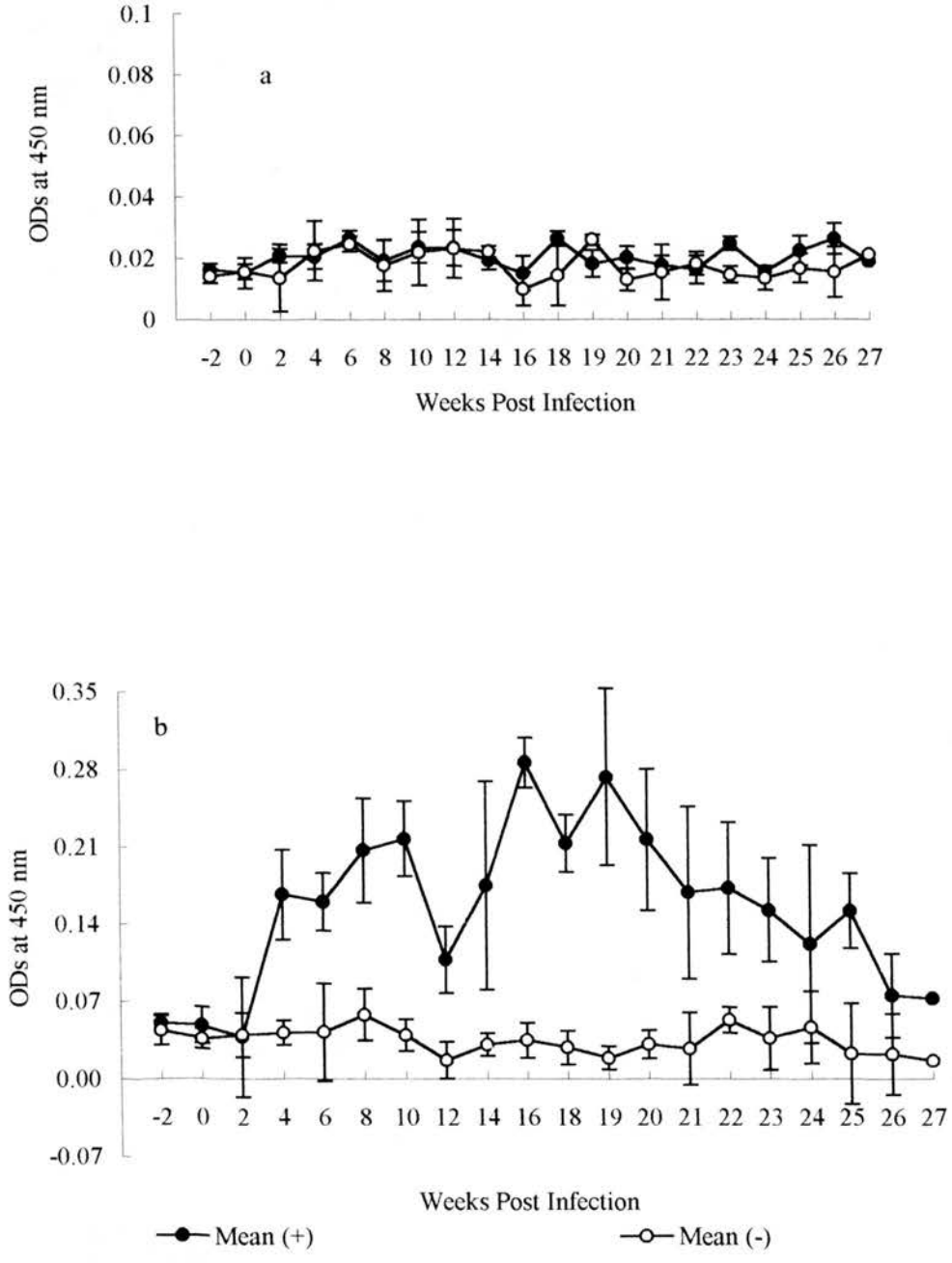


Figure 4.114: The adjusted ELISA OD (450 nm) Mean ± SEM faecal IgG_2 (a) and IgA (b) responses of *F. gigantica* infected sheep (+) and uninfected control sheep (-) to *F. hepatica* Fh-Cathepsin

4.7 FAECAL ANTIBODY RESPONSES TO *F. HEPATICA* GLUTATHIONE S-TRANSFERASE (FH-GST)

4.7.1 Determination of Optimum Assay Condition by Titration.

The optimum assay conditions, i.e. those which optimised the signal to the background ratio, were determined by titration of antigen (Fh-GST) and conjugate for the polyclonal detection system or antigen (Fh-GST), monoclonal antibody and conjugate for the monoclonal antibody based detection system. Titrations were run in duplicates and the mean values calculated. Figures 4.115-4.118: are representative titrations for total Ig and IgA in *F. hepatica* and *F. gigantica* infected sheep and cattle for the two detection systems, polyclonal antibody (Ig) and monoclonal antibody (IgA) detection systems. The selected concentrations for the rest of the assays IgM, IgG2 and IgA for sheep are in Table 4.21 and 4.22.

Chequerboard titration for monoclonal antibodies and conjugate were carried out by diluting these systems in blocking buffer using doubling serial dilution ranging from 2-0.5µg/ml (Antigen), 1:10-1:40 (monoclonal antibody) and 1:1000-1:4000 (conjugate). The chosen antigen concentration, monoclonal antibody and conjugate dilution were used in all subsequent sequential screenings. The faecal samples were used at a strongest possible dilution of 1:1. The two positive sera P1 and P2 were taken from *F. hepatica* or *F. gigantica* infected animals and corresponded week 8-10 (P1) and week 21-22 (P2) post infection for sheep and week 7 (P1) and 32 (P2) for calves. These times post infection were chosen to assess responses at the middle and at the end of the experimental period and to optimise the signal to background ratio. Full data is represented in Appendix tables 4.159-4.163

Table 4.21 Sheep infected with *F. hepatica* or *F. gigantica*: optimal dilution of antigen (μg/ml), faecal sample and conjugate for polyclonal antibody system using *F. hepatica* Glutathione S-Transferase antigen (Fh-GST).

Assay	*F. hepatica*			*F. gigantica*		
	Fh-GST	faeces	Conj.	Fh-GST	faeces	Conj.
Total Ig	1	1:1	1:1000	1	1:1	1:1000
IgM	1	1:1	1:1000	1	1:1	1:1000

Table 4.22 Sheep infected with *F. hepatica* or *F. gigantica*: optimal dilution of antigen (μg/ml), faecal sample, monoclonal antibody (McAb) and conjugate for monoclonal antibody system using *F. hepatica* Glutathione S-Transferase antigen (Fh-GST).

Assay	*F. hepatica*				*F. gigantica*			
	Fh-GST	faeces	McAb	Conj.	Fh-GST	faeces	McAb	Conj.
IgG_1	1	1:1	1:20	1:1000	1	1:1	1:20	1:1000
IgG_2	1	1:1	1:20	1:1000	1	1:1	1:20	1:1000
IgA	1	1:1	1:20	1:1000	1	1:1	1:20	1:1000

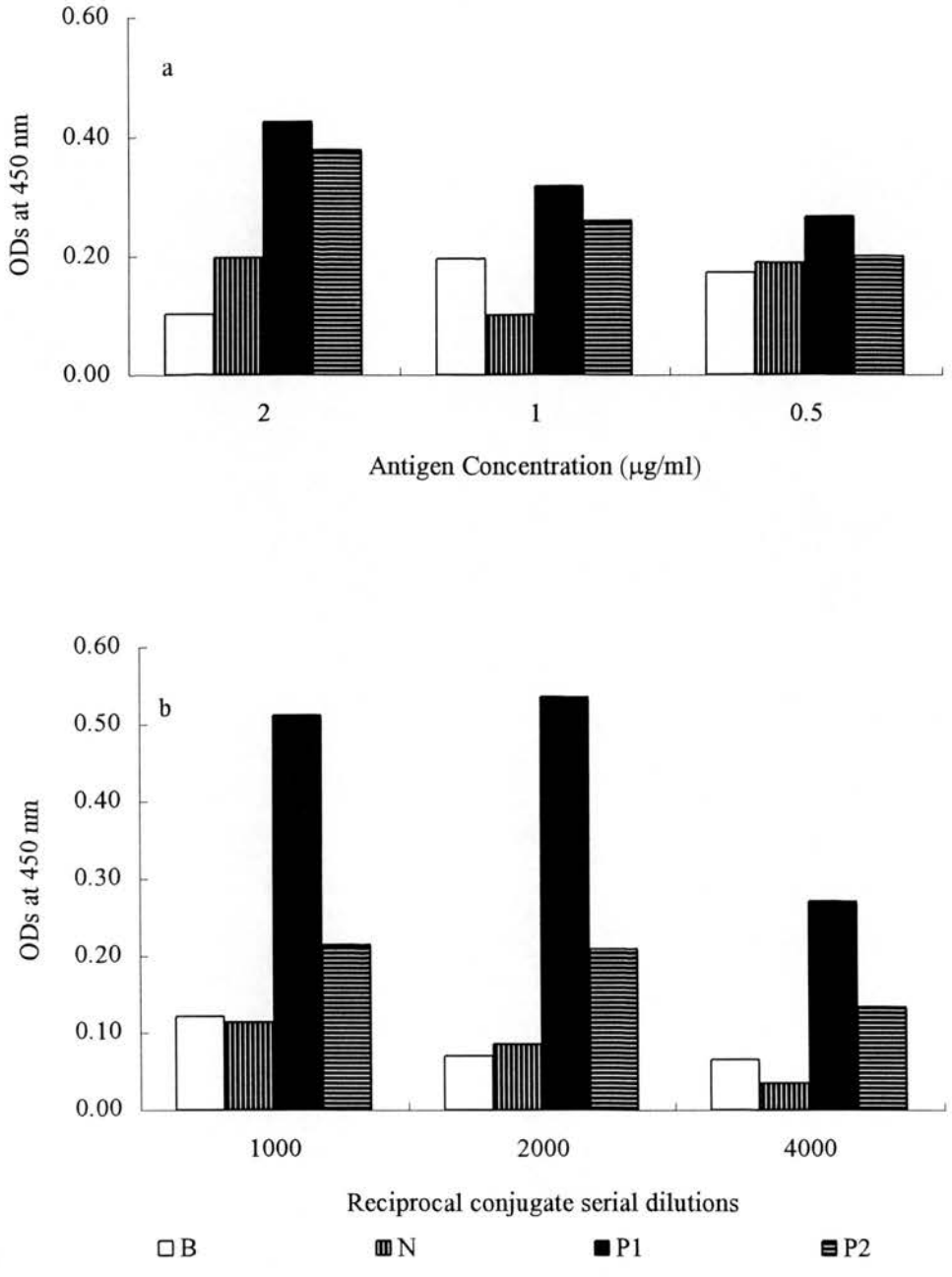


Figure 4.115: Antigen (FhGST) (a) and conjugate (b) titrations for total Ig for *F. hepatica* showing the mean ELISA (450 nm) values obtained from infected sheep and uninfected control sheep for diluent (B), negative (N), positive (P1) and positive (P2)

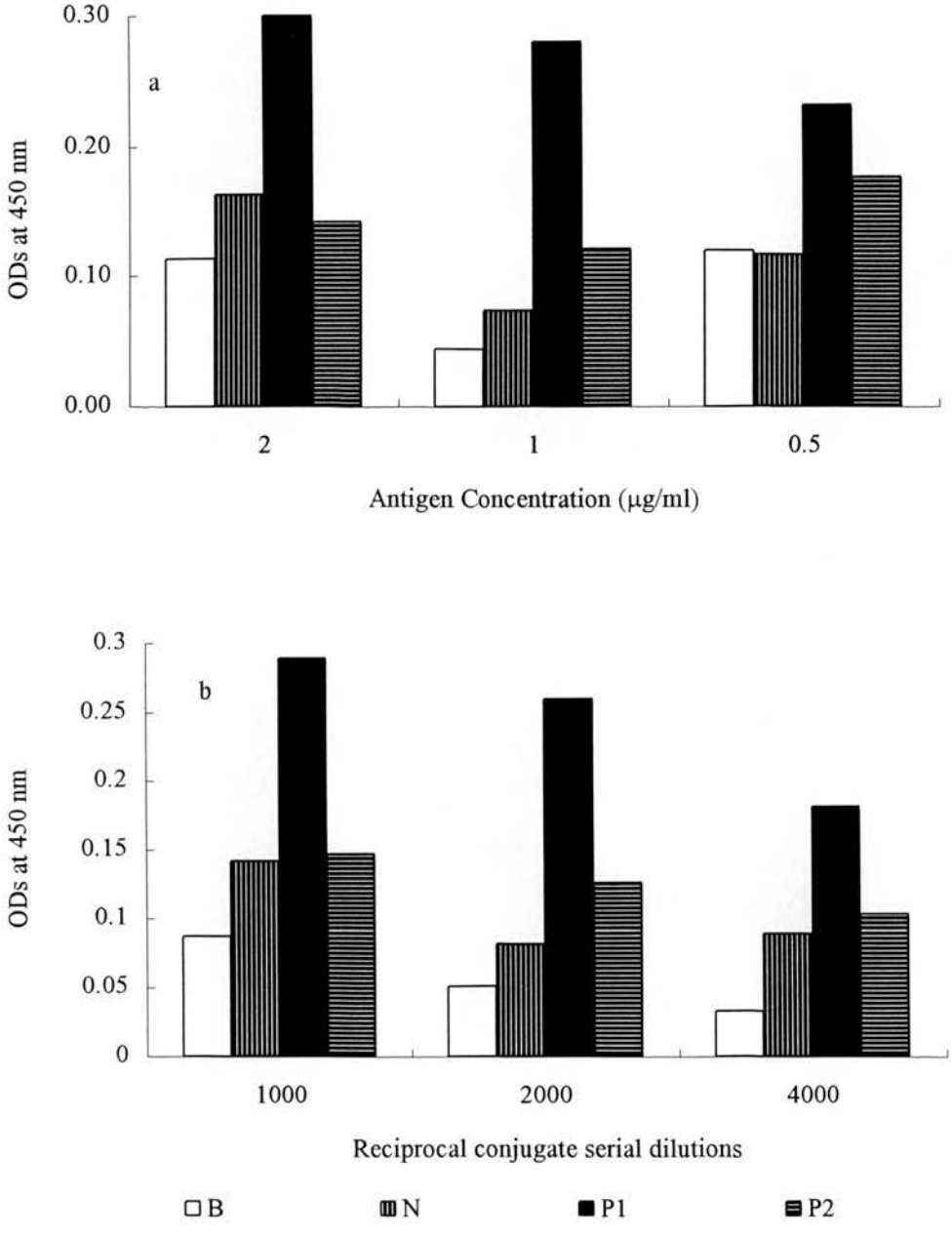


Figure 4.116: Antigen (FgGST) (a) and conjugate (b) titrations for total Ig for *F. gigantica* showing the mean ELISA values obtained from infected sheep and uninfected control sheep for diluent (B), negative (N), positive (P1) and positive (P2)

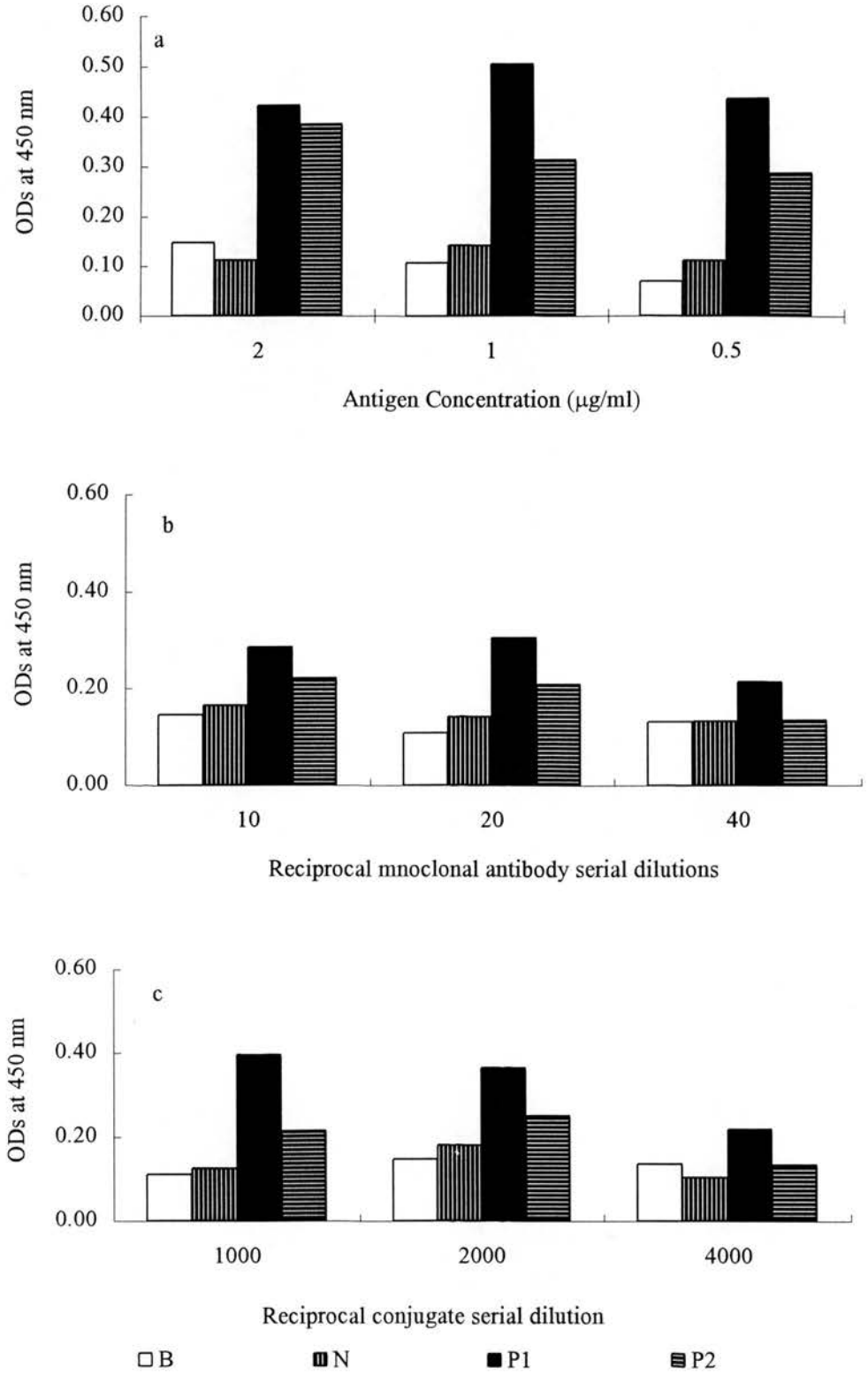


Figure 4.117: Antigen (FhGST) (a), monoclonal antibody (b) and conjugate (c) titrations for faecal IgA for *F. hepatica* infected sheep and uninfected control sheep showing the mean ELISA (450 nm) values obtained for diluent (B) negative (N) positive (P1) and positive (P2)

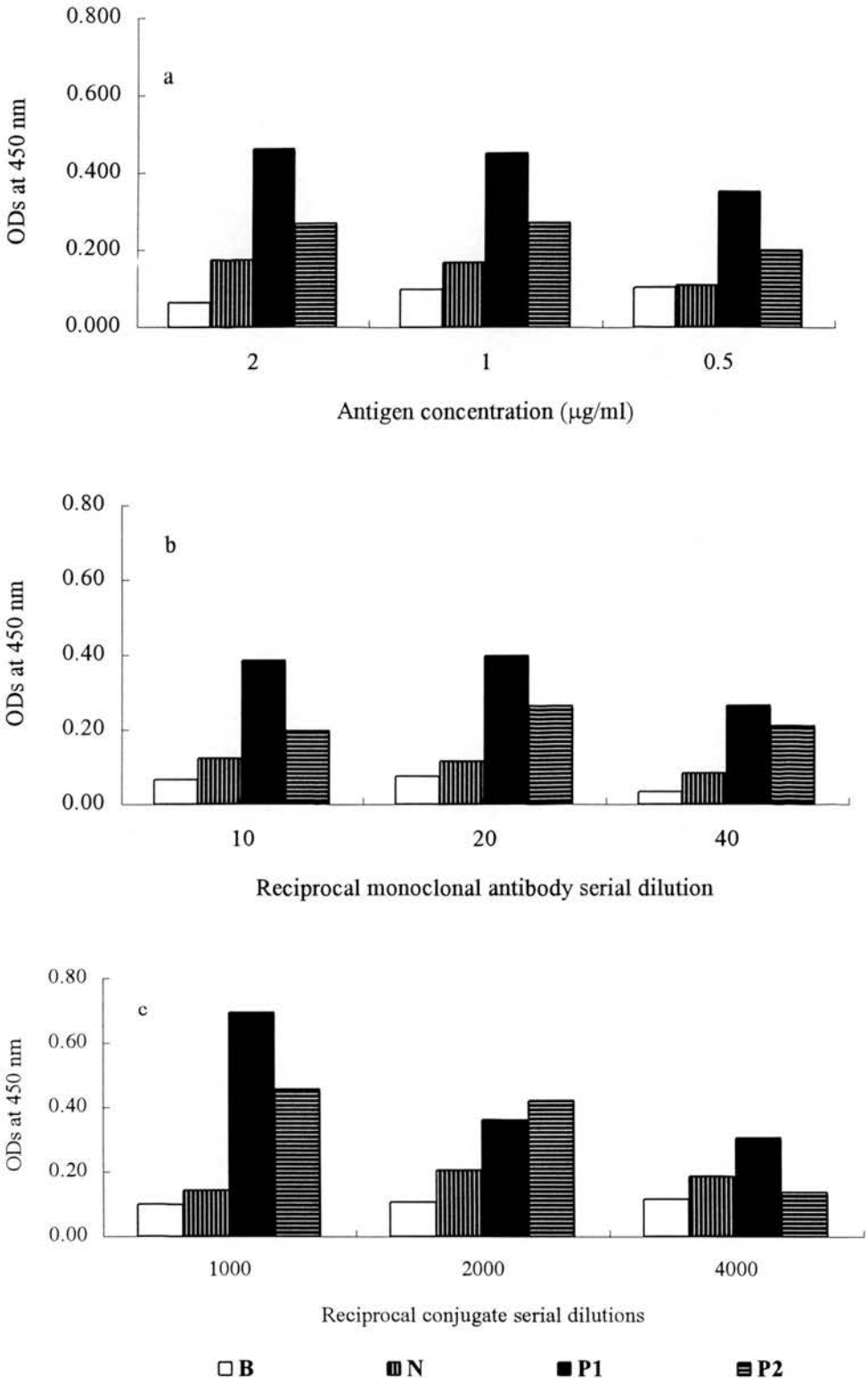


Figure 4.118: Antigen (FgGST) (a), monoclonal antibody (b) and conjugate (c) titration for faecal IgA for *F. gigantica* infected sheep and uninfected sheep showing the mean ELISA (450 nm) values obtained for diluent (B) negative (N) positive (P1) and positive (P2)

4.7.2 Experiment 1: *F. hepatica* (Peruvian and British strain) Infection in Sheep

Only total faecal Ig and IgA antibody responses to *F. hepatica* Glutathione S-Transferase antigen (Fh-GST) were detected in the infected sheep. There were no noticeable faecal IgG_1, IgM, and IgG_2 response in the infected sheep (Figures 4.119a-4.120b. The adjusted data is shown in Appendix Tables 4.164-4.166

All the infected sheep showed an early (2 wpi.) increase in total faecal Ig levels peaking 6-7 wpi. OD values then started to fall by 8 wpi. only to start rising again 12 wpi. showing a clear biphasic IgA response to Fh-GST. The first peak was higher than the second peak.

There was early, 2 wpi., faecal IgA response to Fh-GST by sheep 6 and 9. There was late response by sheep 7 and 10. It is clear that IgA was the main Ig response to *F. hepatica* infection in these sheep.

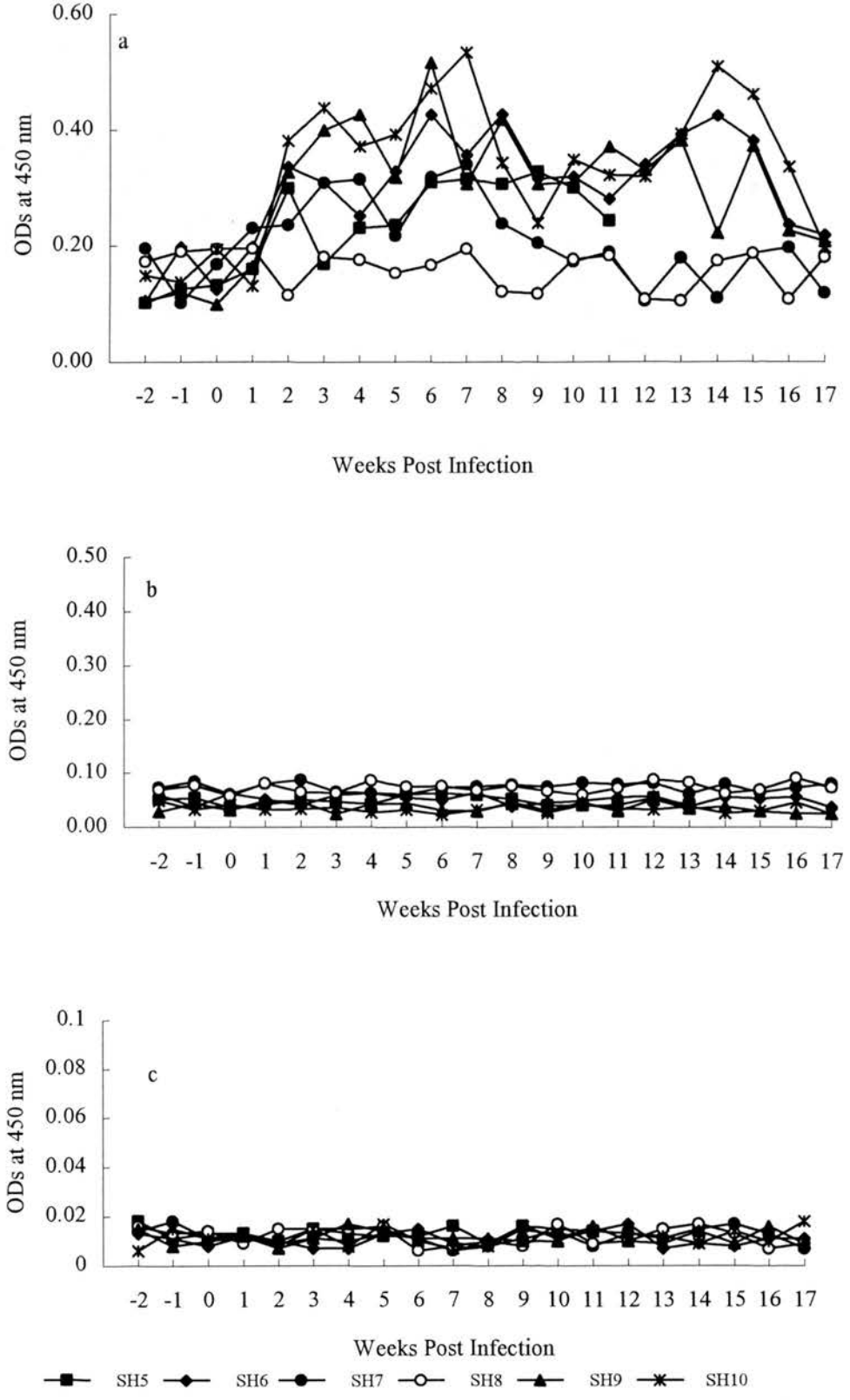


Figure 4.119: The adjusted ELISA OD (450 nm) faecal total Ig (a), IgG_1 (b) and IgM (c) responses of *F. hepatica* infected sheep 5, 6, 7, 9 and 10 and uninfected control sheep 6 to *F. hepatica* Glutathion S-Transferase (FhGST)

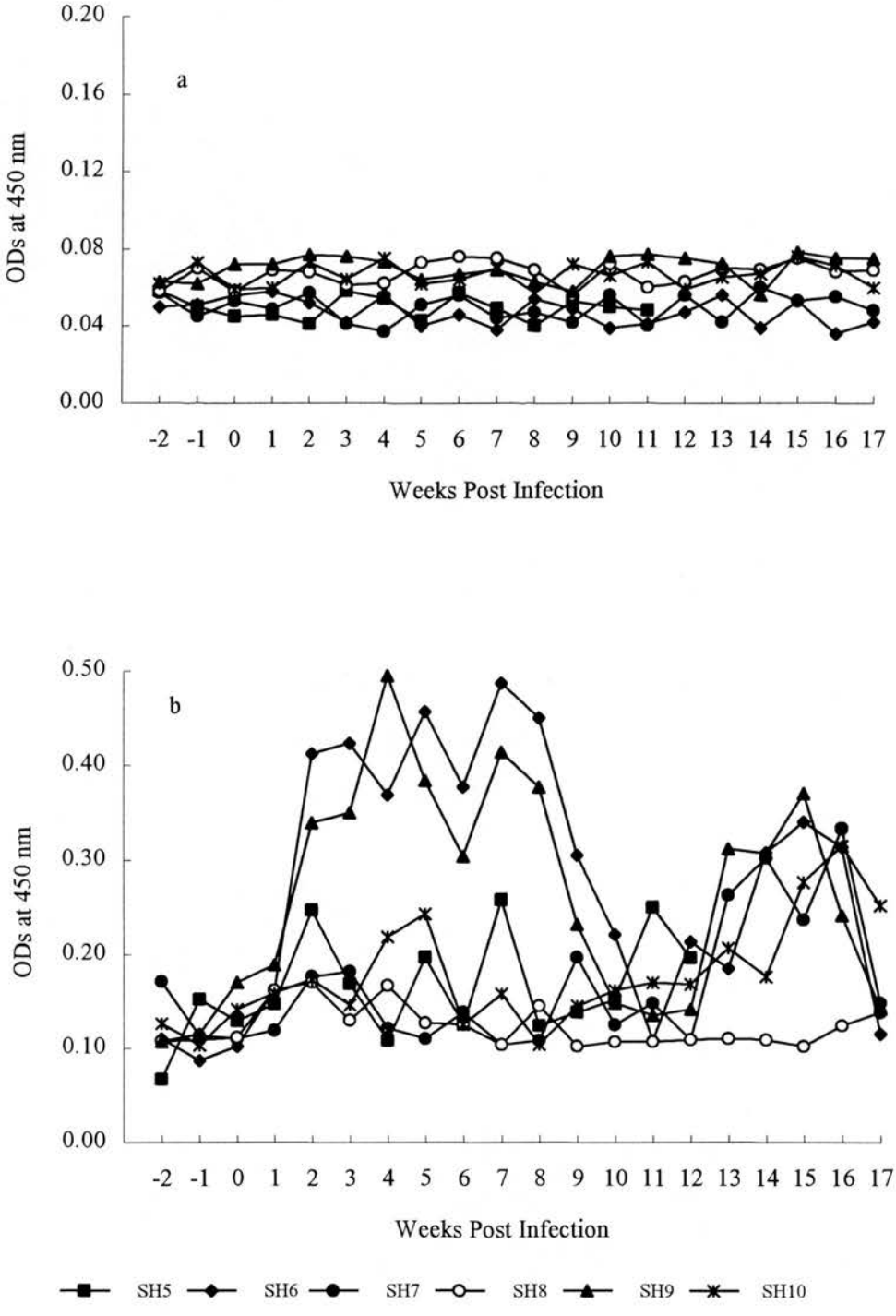


Figure 4.120: The adjusted ELISA OD (450 nm) faecal IgG_2 (a) and IgA (b) responses of F. hepatica infected sheep 5, 6, 7 and 9 and 10 uninfected contro sheep 8 to *F. hepatica* Glutathion S-Transferase (FhGST)

4.7.3 Experiment 3: *F. gigantica* (Kenyan strain) Infection in Sheep

Only total faecal Ig and IgA antibodies response was detected to Fh-GST in the samples from *F. gigantica* infected animals. The subclasses IgM, IgG_1 and IgG_2 were negative (Figures 4.121a-4.122b.

The infected sheep showed an early increase in total Ig levels, 4 wpi. and peaked at 19 wpi. The mean OD levels started to drop from 22 wpi. By the end of experiment however the mean OD values in the infected sheep were almost at the same level as that of uninfected control sub group. The response was by phasic.

There was an early, 4 wpi., sharp faecal IgA response to Fh-GST *F. gigantica* infected sheep with the peak 16 wpi. followed by a drop 20 wpi.

The mean ELISA ODs of adjusted data are presented in Appendix Tables 4.167-4.170 and adjusted data for the responses by the faecal antibodies in cattle to the three antigens is shown in appendix table 4.171.

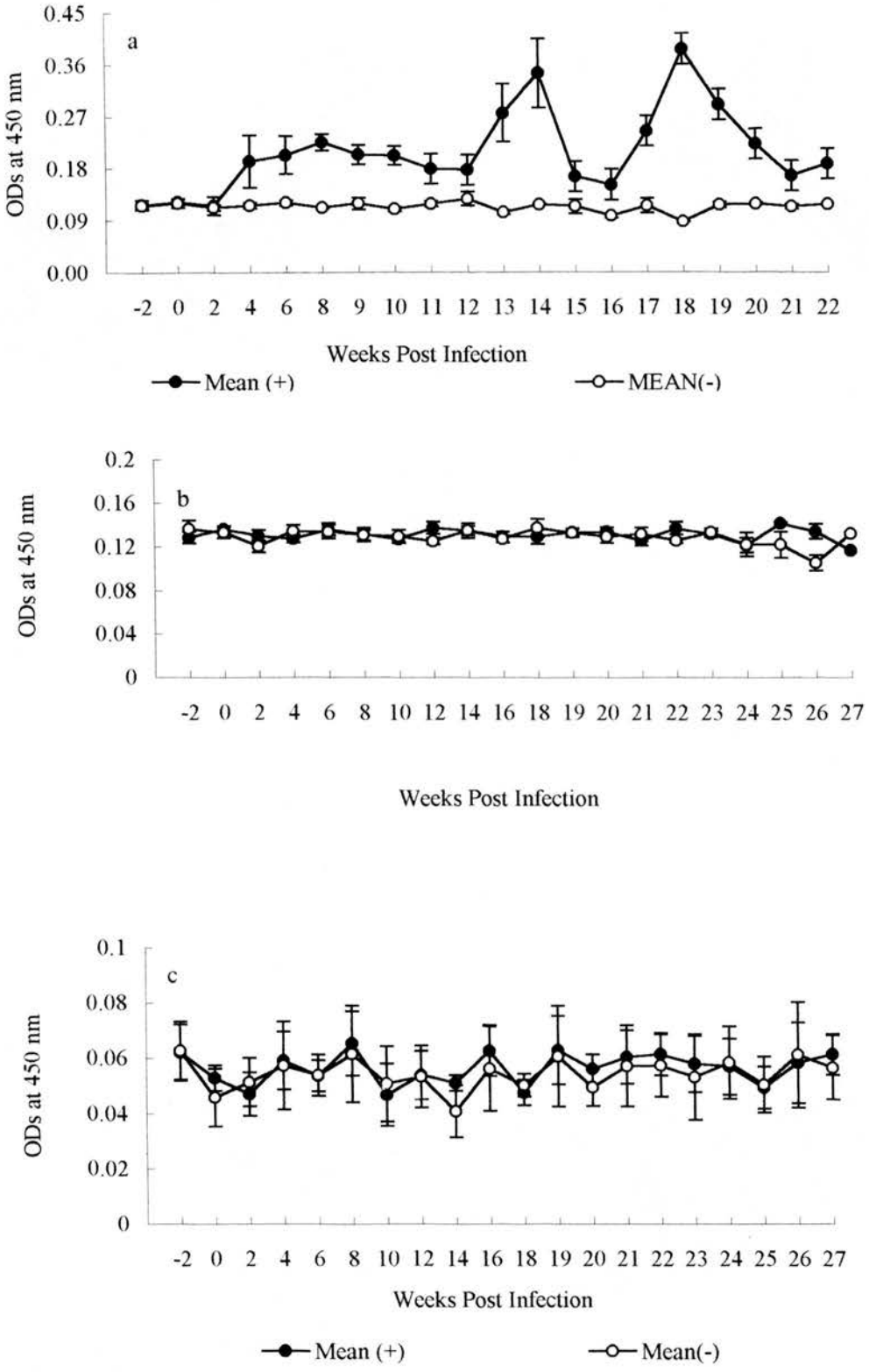
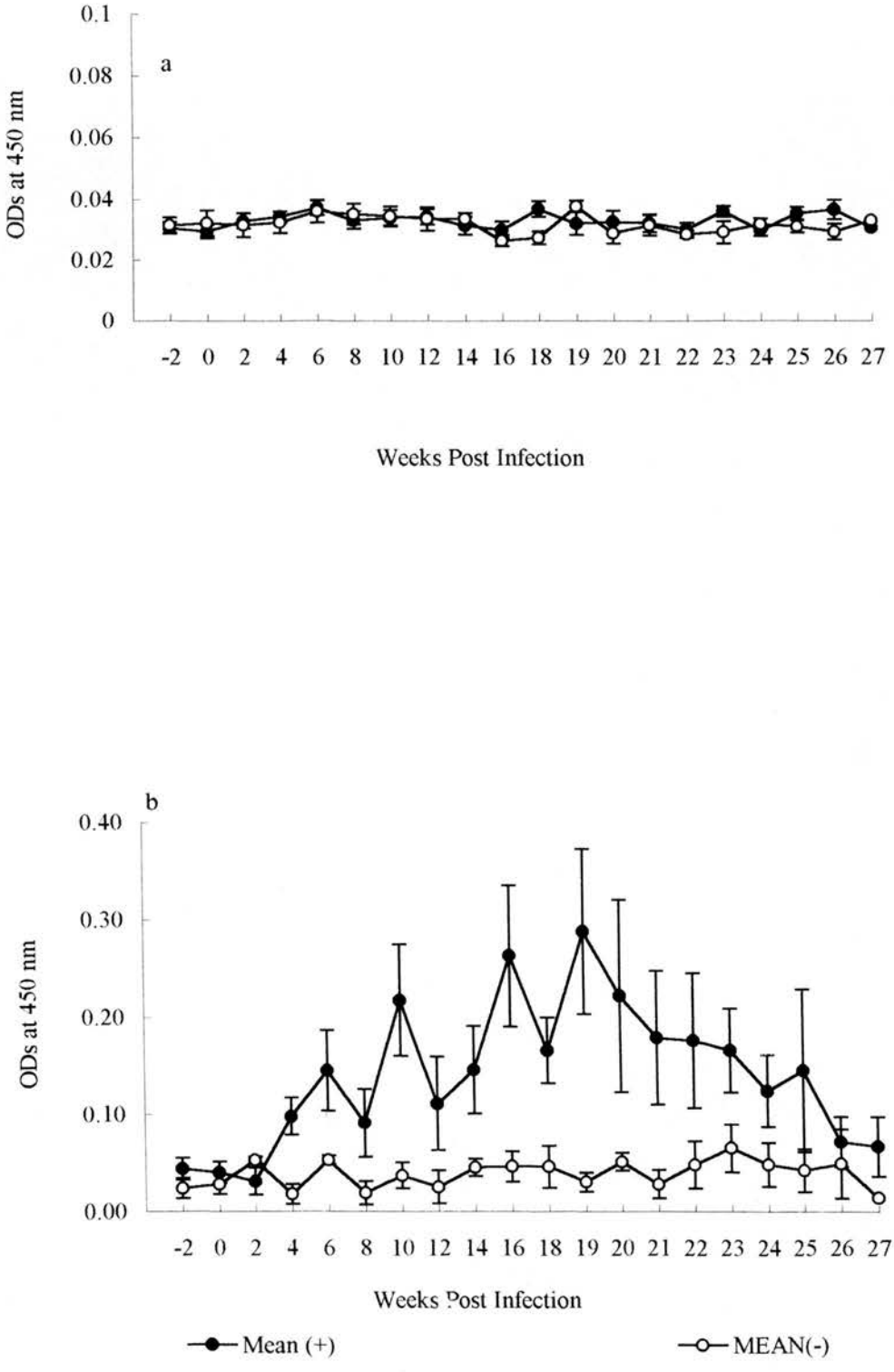


Figure 4.121: The adjusted ELISA OD (450 nm) mean ± SEM faecal total Ig (a), IgG_1 (b) and IgM (c) responses of *F. gigantica* infected sheep (+) and uninfected control sheep (-) to *F. hepatica* Glutathion S-Transferase (FhGST)



Figigure 4.122: The adjusted ELISA OD (450 nm) mean ± SEM faecal IgG2 (A) and IgA (b) responses of *F. gigantica* infected sheep (+) and uninfected control sheep (-) to *F. hepatica Glutathion S-Transferase* (FhGST).

4.8 IMMUNOCHEMISTRY

4.8.1 The Protein Concentration of the *Fasciola spp.* Excretory/Secretory products

The adult *F. hepatica* and *F. gigantica* E/S products protein concentration measured according to Warburgh and Christian (1941) is shown in Table 4.23. In all cases the E/S with highest protein concentration 462 µg/ml and 196 µg/ml were used for Western Blotting. The adult *F. hepatica* and *F. gigantica* E/S products used throughout these studies were produced by liver fluke from experimentally infected sheep.

Table 4.23: The protein concentration of excretory/secretory products from *F hepatica* (British strain) and *F. gigantica* (Kenya strain) collected from livers of experimentally infected sheep.

	Period Cultured (Hours)	Protein Concentration (µg/ml)
Fh-E/S Products	0-24	237
	24-48	462
Fg-E/S Products	0-24	186
	24-48	196

The silver stained protein profile of the E/S products of adult *F. hepatica* and *F. gigantica* are presented in figure 4.123. The different batches synthesised similar polypeptides. However, due to low concentration, more polypeptides were in the 0-48 hours preparation of both Fh-E/S and Fg-E/S than in 0-24 hours E/S. In reduced sample the 24 hours Fh-E/S contained proteins of molecular weights ranging from 17 to 181 kDa, 48 hours Fh-E/SP ranging from 15 to 205 kDa, 24 hours Fg-E/S from 17 to 205 kDa and 48 hours Fg-E/S 17 to 205 kDa.

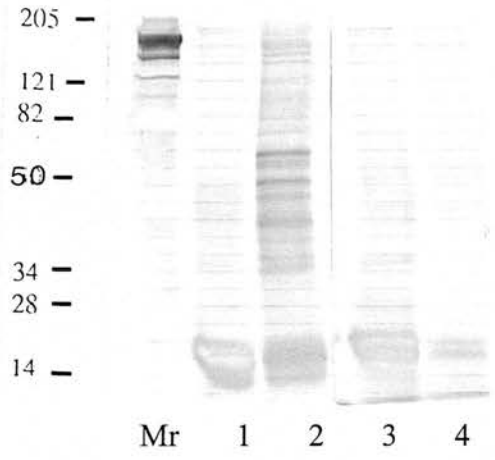


Figure 4.123 Silver stain of 0-24 hours *F. hepatica* E/S Products (track 2), 24-48 hours *F. hepatica* E/S Products (track 3) 0-24 hours *F. gigantica* E/S Products (track 4) and 24-48 (track 3 and 5) hours and 24-48 hours *F. gigantica* E/S Products (track 5) following separation using SDS-PAGE. Samples were all run under reduced conditions. The protein of the molecular weight $x10^3$ markers are on the left.

4. 13.2 Experiment 1 & 2 *F. hepatica* Infected Sheep

Titration

The results to determine the optimum serum and biotinulated rabbit anti-sheep conjugate dilutions for use in Western Blotting are shown in Figure 4.124. The 24-48 hours adult *F. hepatica* E/S was selected for the assay. The undiluted 24-48 hours Fh-E/S solutions concentration of 462 µg/ml was found to be sufficient for assay. The test sera of 1/50 and 1/2000 biotinulated donkey anti-sheep IgG produced the most clear reaction and therefore was used in all subsequent Western Blotting assays in *F. hepatica* infected sheep. Streptavidin alkaline phosphatase was used as recommended by the manufacturers (1/3000). The sera of infected sheep were able to recognise more than five (5) molecules ranging from 14 kDa to 142 kDa.

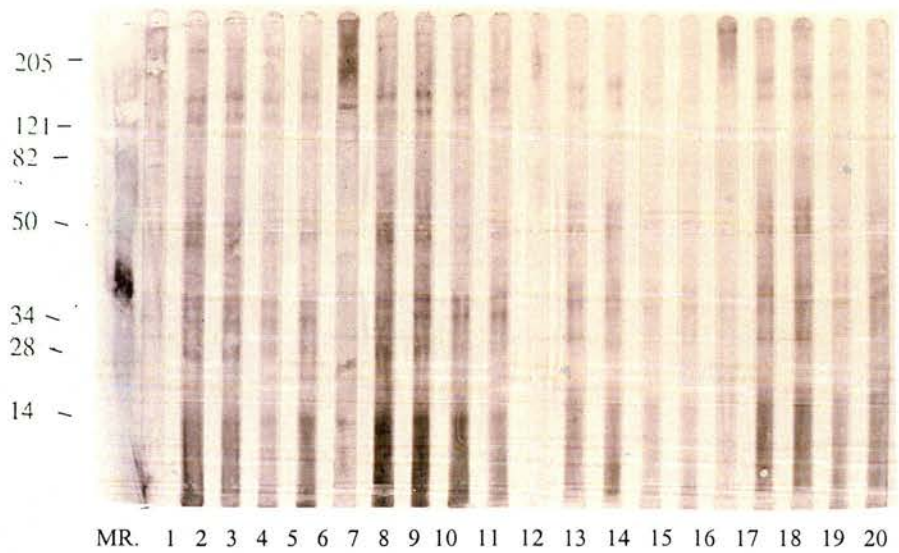


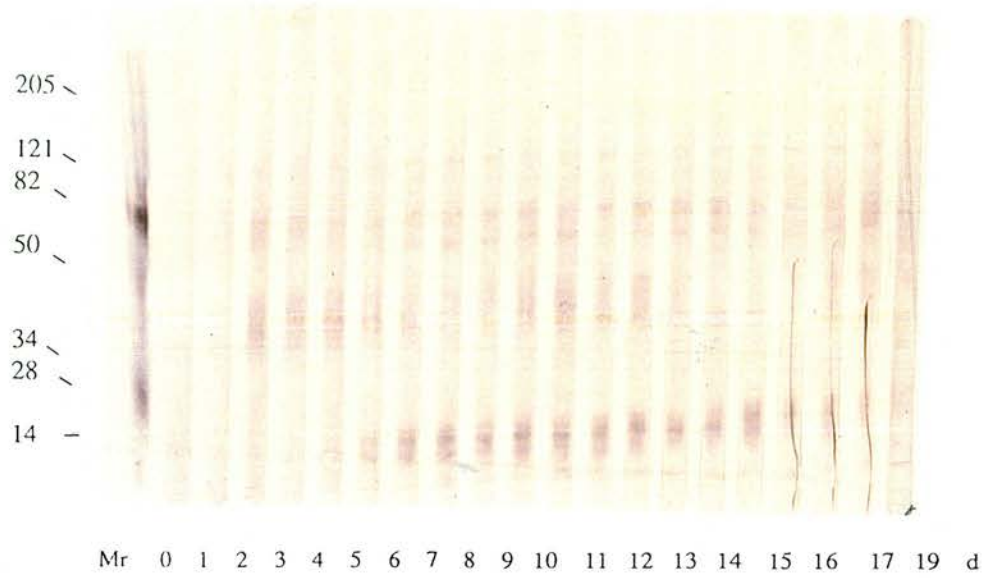
Figure 4.124 Titration to determine the optimum *F. hepatica* infected sheep serum and biotin labelled anti-sheep conjugate dilutions for immunostaining electroblotted 24-48 hours *F. hepatica* E/S products under reducing conditions. The position of molecular weight markers (Mr) are shown on the left and below are lane numbers.

Lanes 1-, 6, 11 and 16	4% NRS/PBS/Tween 20
Lanes 2-5 and 12-15	1:50 serum from infected sheep
Lanes 7-10 and 17-20	1:100 serum from infected sheep
Lanes 1-10	1:2000 Donkey anti-sheep conjugate
Lanes 11-20	1:4000 Donkey anti-sheep conjugate

Western Blotting

There was a common antigen recognition in all *F. hepatica* infected sheep serum although the time and duration of the recognition of individual antigen differed from sheep to sheep. By 3-5 wpi. antigens of 54 kDa and 79 kDa were recognised by all the infected sheep serum. A 14 kDa molecule doublet was recognised by all the infected sheep serum, (sheep 7, 9, 10, 24, 26, 28 and 30), starting from 7-8 wpi. A high molecular weight of 134 kDa was recognised by serum from sheep 7 infected with British *F. hepatica* and 10 infected with Peruvian *F. hepatica*. The results for uninfected control sheep were negative so were the prebleeds of infected animals. The representative figures are shown below (Figures 4.125-4.127).

(a)



(b)

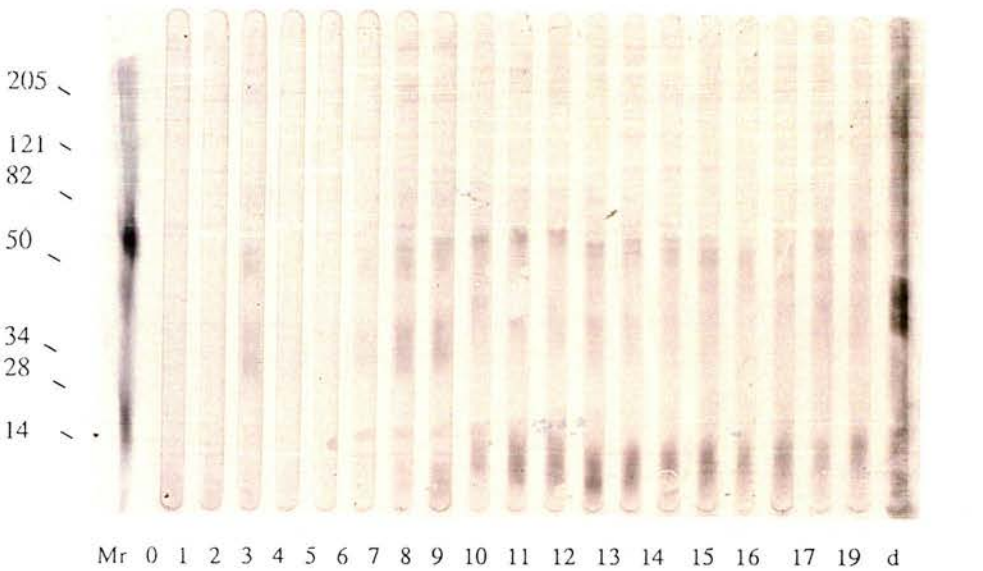
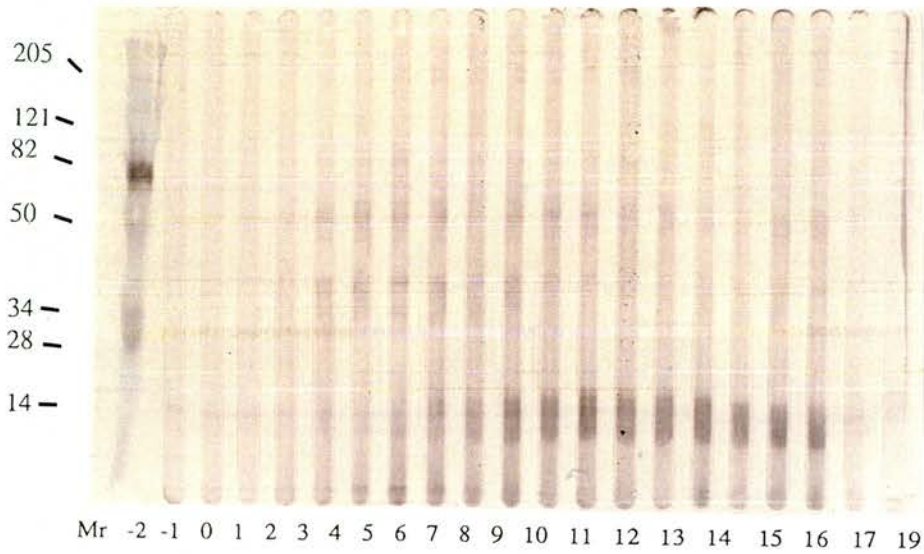


Figure 4.125 (a & b) The 24-48 hours Fh-E/S recognition by sera of *F. hepatica* infected sheep 7 (a) and sheep 9 (b) under reducing condition. The position of molecular weight markers (Mr) pre-bleed (0) and diluent (d) and the numbers at the bottom represent weeks post infection (wpi.) and the molecular weights are shown on the left hand side of the figures.

(a)



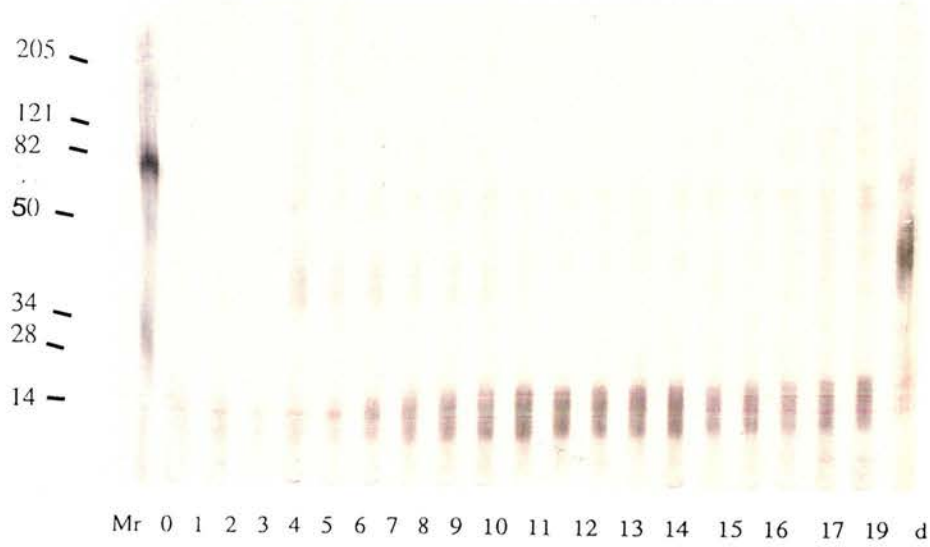


Figure 4.126 (a & b) The 24-48 hours Fh-E/S recognition by sera of *F. hepatica* infected sheep 10 (a) and 28 (b) under reducing condition. The position of molecular weight markers (Mr) pre-bleed (-2) and diluent (-1) and the numbers at the bottom represent weeks post infection (wpi.) and the molecular weights are shown on the left hand side of the figures.

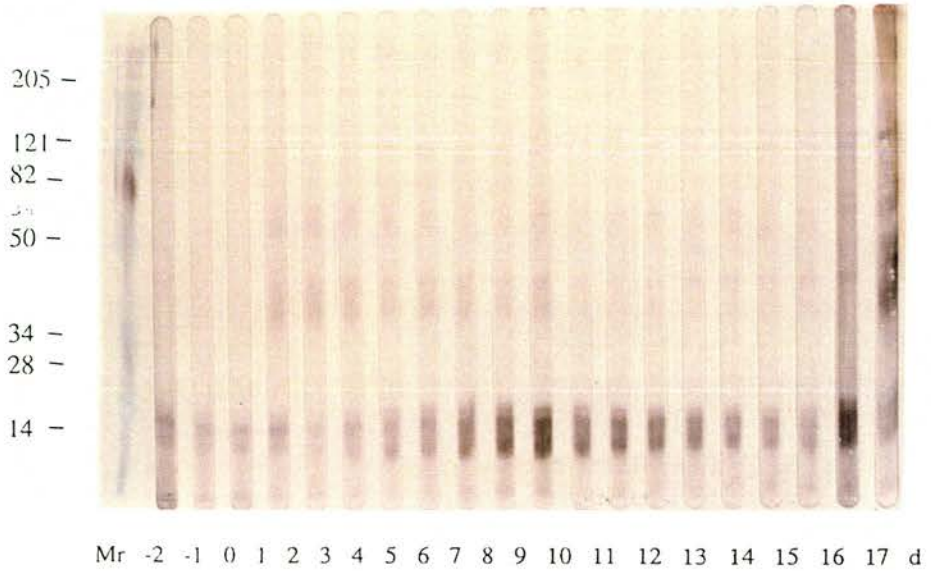


Figure 4.127 The 24-48 hours Fh-E/S recognition by sera of *F. hepatica* infected sheep 30 under reducing condition. The position of molecular weight markers (Mr) pre-bleed (-1 & -2) and diluent (d) and the numbers at the bottom represent weeks post infection (wpi.) and the molecular weights are shown on the left hand side of the figures.

4. 8.3 Experiments 3 & 4: *F. gigantica* Infected Sheep

Titration

The results to determine the optimum, serum and biotinulated rabbit anti-sheep conjugate, dilutions for use in immunostaining electroblotting are shown in Figure 4.128. The 24-48 hours adult *F. gigantica* E/S was selected for the assay. The undiluted 24-48 hours Fg-E/S solutions concentration of 196 μg/ml was found to be sufficient for assay. The test sera of 1/50 and 1/2000 biotinulated rabbit anti-sheep Ig produced most clear reaction and therefore was used in all subsequent western blotting assays in *F. gigantica* infected sheep. Streptavidin alkaline phosphatase was used as recommended by the manufacturers (1/3000). The sera of infected sheep recognise 14 kDa doublet antigen.

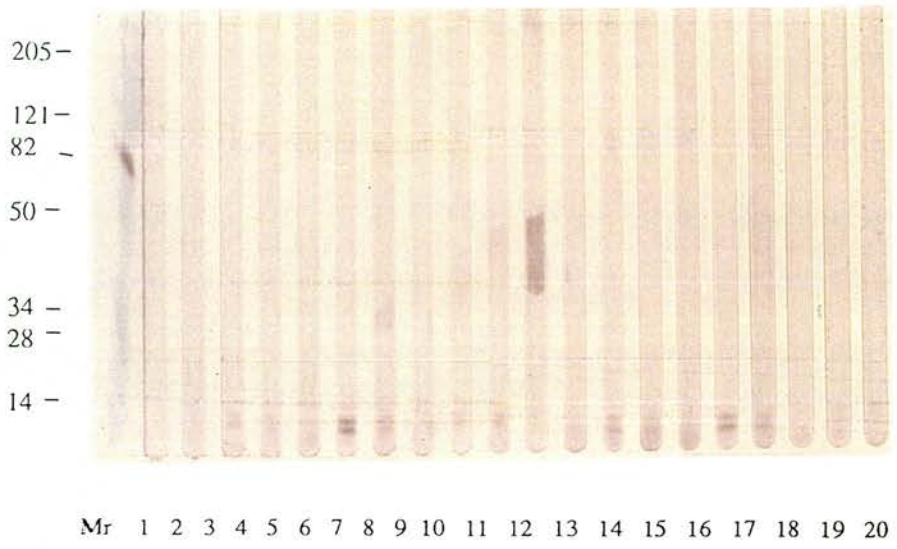


Figure 4.128 Titration to determine the optimum *F. gigantica* infected sheep serum and biotin labelled anti-sheep conjugate dilutions for immunostaining electroblotted 24-48 hours *F. gigantica* E/S products under reducing conditions. The position of molecular weight markers (Mr) are shown on the left.

Lanes 1-, 6, 11 and 16	4% NRS/PBS/Tween 20
Lanes 2-5 and 12-15	1:50 serum from infected sheep
Lanes 7-10 and 17-20	1:100 serum from infected sheep
Lanes 1-10	1:2000 Donkey anti-sheep conjugate
Lanes 11-20	1:4000 Donkey anti-sheep conjugate

Western Blotting

There was a common antigen recognition in all *F. gigantica* infected sheep serum although the time and duration of the recognition of individual antigens differed from sheep to sheep. By 3-5 wpi., antigens of 14 kDa was recognised by all the infected sheep sera. Other molecular weights recognised after patency by the *F. gigantica* infected sheep include a 152 kDa as recognised by sheep 12, 25 and 29 and 88 kDa as recorded by sheep 14 and 29. The results of uninfected control were similar to those of pre-bleeds of the infected experimental animals. The two Western Blotting figures are a representative the antigen recognition pattern of all *F. gigantica* infected sheep serum. The representative figures are shown below (Figures. 4.129 (a & b).

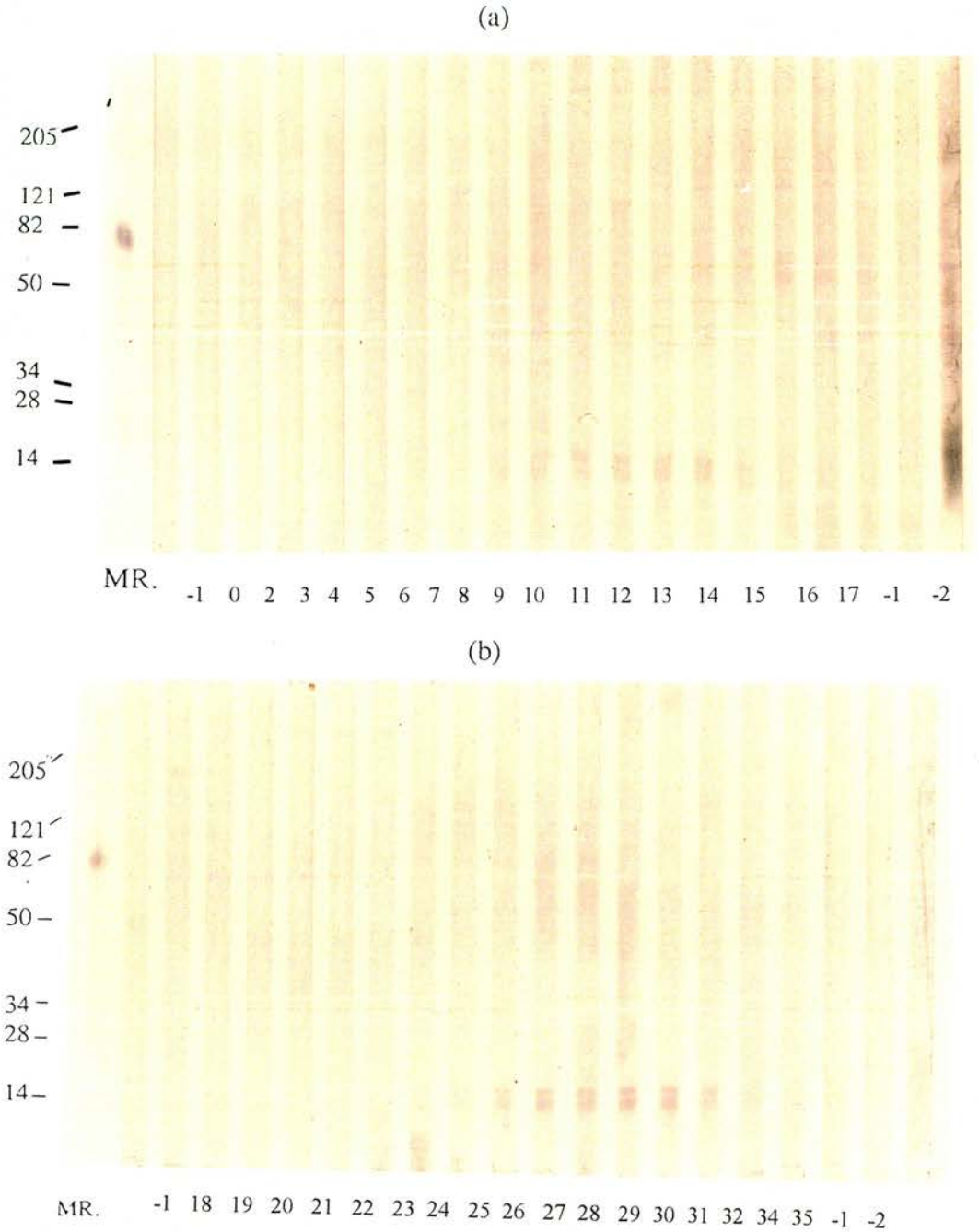


Figure 4.129 (a & b) The 24-48 hours Fg-E/S recognition by sera of *F. gigantica* infected sheep 11 (a) and 12 (b) under reducing condition. The position of molecular weight markers (Mr) pre-bleed (-1) and diluent (d) and the numbers at the bottom represent weeks post infection (wpi.) and the molecular weights are shown on the left hand side of the figures.

4. 8.4 Experiment 5: *F. hepatica* Infected Cattle

Titration

The results to determine the optimum serum and biotinulated goat anti-bovine conjugate dilutions for use in immunostaining electroblotting are shown in Figure 4.130. The 24-48 hours adult *F. gigantica* E/S was selected for the assay. The undiluted 24-48 hours Fh-E/S solutions concentration of 462 µg/ml was found to be sufficient for assay. The test sera of 1/50 and 1/2000 biotinulated bovine anti-bovine Ig produced most clear reaction and therefore was used in all subsequent western blotting assays in *F. hepatica* infected sheep. Streptavidin alkaline phosphatase was used as recommended by the manufacturers (1/3000). Titration results for *F. hepatica* infected sheep showed 7 prominent molecules with the majority banding between 69 kDa and 40 kDa. The other two were one measuring 116 kDa and the other large molecule 134 kDa as shown in Figures: 4.130 There were less than 8 visible antigens in *F. hepatica* infected calves during the titration.

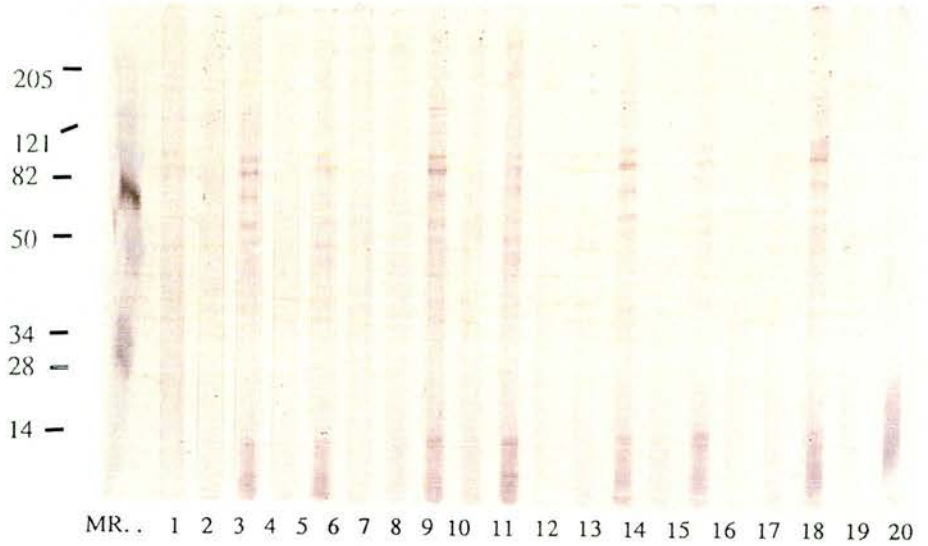


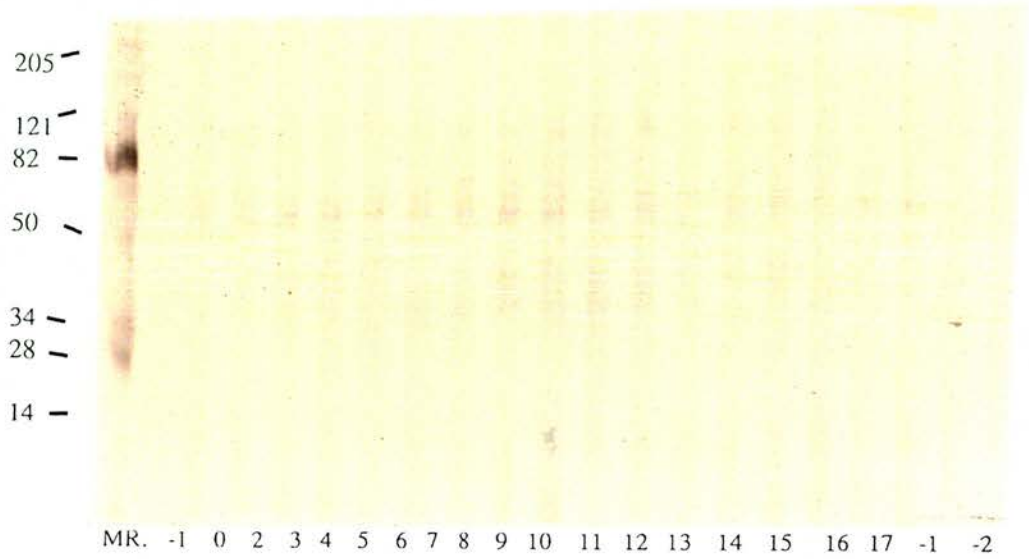
Figure 4.130 Titration to determine the optimum *F. hepatica* infected cattle serum and biotin labelled anti-bovine conjugate dilutions for immunostaining electroblotted 24-48 hours *F. hepatica* E/S products under reducing conditions. The position of molecular weight markers (Mr) are shown on the left.

Lanes 1-, 6, 11 and 16 4% NGS/PBS/Tween 20
Lanes 2-5 and 12-15 1:50 serum from infected sheep
Lanes 7-10 and 17-20 1:100 serum from infected sheep
Lanes 1-10 1:2000 Goat anti-sheep conjugate
Lanes 11-20 1:4000 Goat anti-sheep conjugate

Western Blotting

The was a lot of variation in response to Fh-E/S in calves of these animals (Figures: 4.131-4.132). Calf 14c first recognised 142 kDa 7 wpi., other molecules included a 40 kDa 19 wpi. and 62 kDa after 20 wpi., Calf 15c on the other hand recognised more molecules than calf 14c namely a 142 kDa doublet, 60 and 40 kDa by 7 wpi. and 10 wpi. a 14 kDa was also recognised. Most of these antigens were less prominent by 37 wpi. The antigen with 142 molecular weight was the most interesting in this calf with challenge infection, after appearing 7 wpi. this molecule faded away by 18 wpi. only to reappear three weeks after challenge infection. Calf 23c serum recognised all the molecules calf 15 did and after challenge similar response to molecule 142 was shown. The results of uninfected control calf 26c were similar to those of pre-bleeds of the infected experimental animals. Calves 34c and 45c clearly recognised a 14 kDa molecule by 6 wpi. Another less clear molecule recognised by these Peruvian *F. hepatica* infected cattle is a 142 kDa molecule.

(a)



(b)

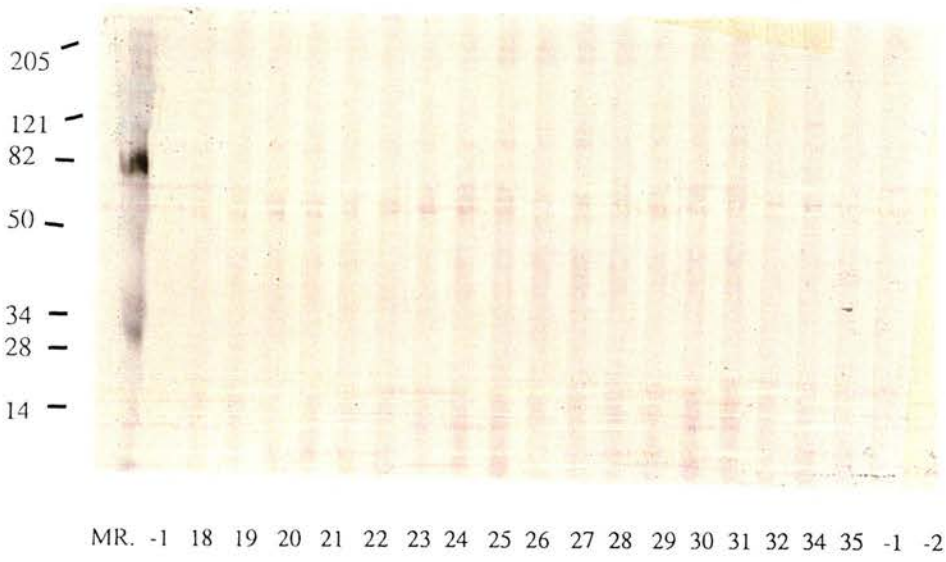
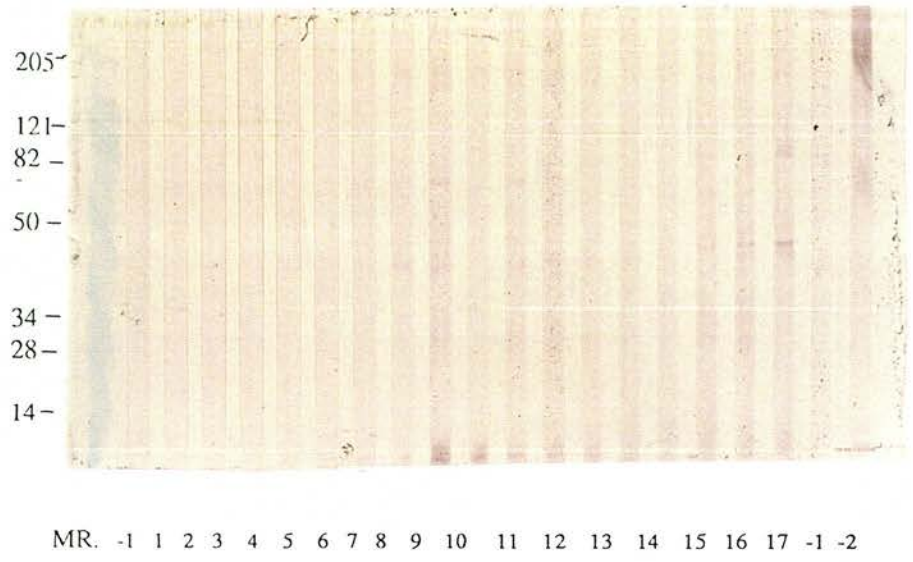


Figure 4.131 (a & b) The 24-48 hours Fh-E/S recognition by sera of *F. hepatica* infected calf 15c -1 to 17 wpi. (a) and 20 to 32 wpi.(b) under reducing condition. The position of molecular weight markers (Mr) pre-bleed (n) and diluent (d) and the numbers at the bottom represent weeks post infection (wpi.) and the molecular weights are shown on the left hand side of the figures.

(a)



(b)

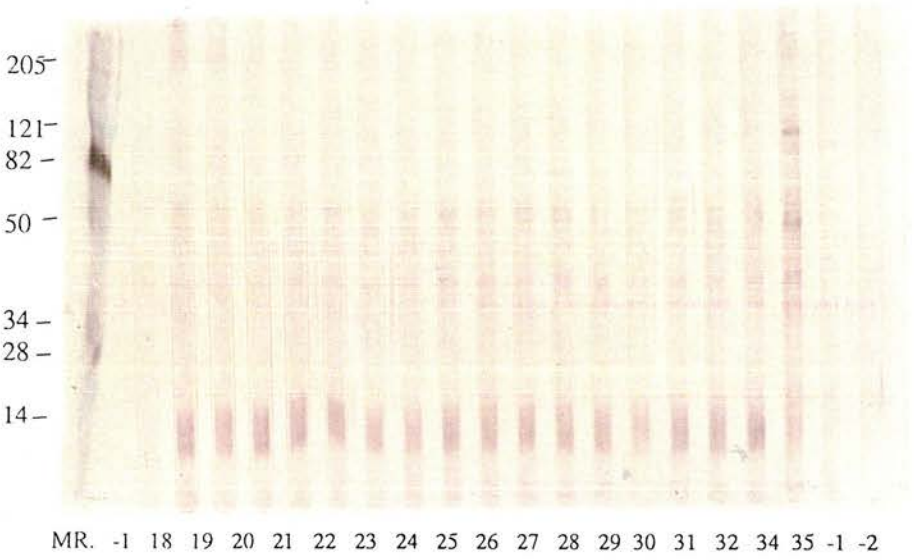


Figure 4.132 (a & b) The 24-48 hours Fh-E/S recognition by sera of *F. hepatica* infected calf 23c, -1 to 17 wpi. (a) and 20 to 32 wpi.(b) under reducing condition. The position of molecular weight markers (Mr) pre-bleed (-1) and diluent (-2) and the numbers at the bottom represent weeks post infection (wpi.) and the molecular weights are shown on the left hand side of the figures.

4.8.5 Experiment 6: *F. gigantica* Infected Calves

Titration

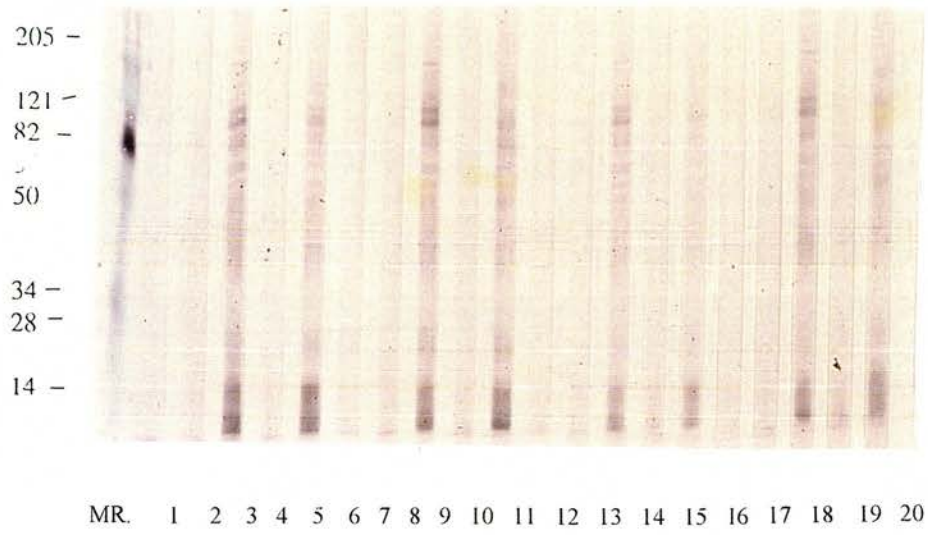
The results to determine the optimum, serum and biotinulated goat anti-bovine conjugate, dilutions for use in immunostaining electroblotting are shown in Figure 4.133.

The 24-48 hours *F. gigantica* was selected for the assay because of it's clearer banding results obtained by SDS-PAGE/Silverstain. The undiluted 24-48 hours Fg-E/S solutions concentration of 196 μg/ml was found to be sufficient for assay for *F. gigantica* infected calves. The test sera of 1/50 and 1/2000 biotinulated goat anti-bovine Ig produced most prominent antigens therefore was used in all subsequent western blotting assays. A one to three thousand parts (1/3000) dilution of Streptavidin alkaline phosphatase was used as recommended by the manufacturers. Titration results show recognition of more than ten molecules starting from 11 to 142 kDa molecular weight.

Western Blotting

There was clear change in the antigen recognition pattern of infected calves throughout the course of infection. In the early stage antigens of doublet 134 kDa was recognised first 4-5 wpi. A different set of antigens, doublet 14 and doublet 60 kDa Mwt were recognised 8-9 wpi. The results of the uninfected control were similar to those of pre-bleeds of the infected experimental animals. The results of individual infected calves are shown in figures 4.134a-4.136b .

a



b

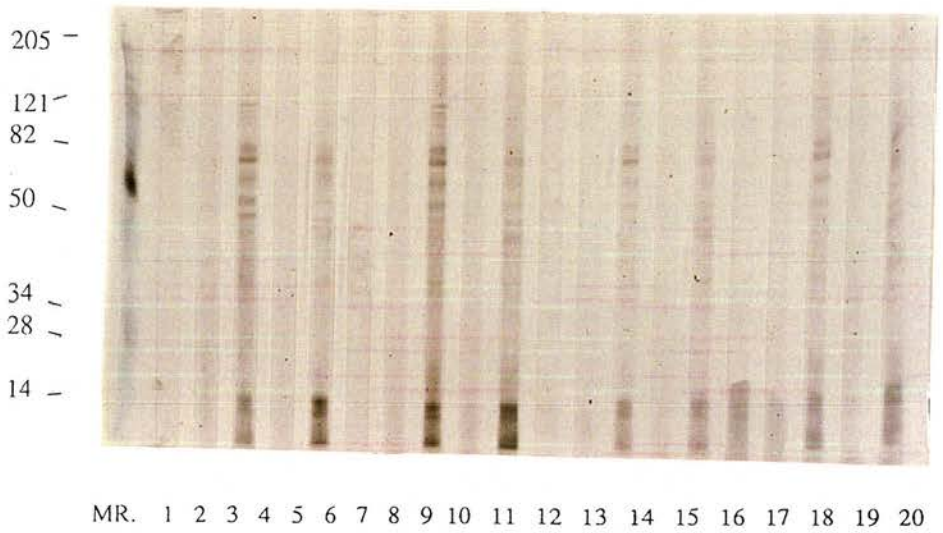


Figure 4.133 (a & b) Titration to determine the optimum *F. gigantica* infected cattle serum and biotin labelled goat anti-bovine conjugate dilutions for immunostaining electroblotted 24-48 hours *F. gigantica* E/S products under unreducing (a) reducing (b) conditions. The position of molecular weight markers (Mr) are shown on the left and on the bottom is the number of lanes.

Lanes 1-, 6, 11 and 16	4% NGS/PBS/Tween 20
Lanes 2-5 and 12-15	1:50 serum from infected sheep
Lanes 7-10 and 17-20	1:100 serum from infected sheep
Lanes 1-10	1:2000 Donkey anti-sheep conjugate
Lanes 11-20	1:4000 Donkey anti-sheep conjugate

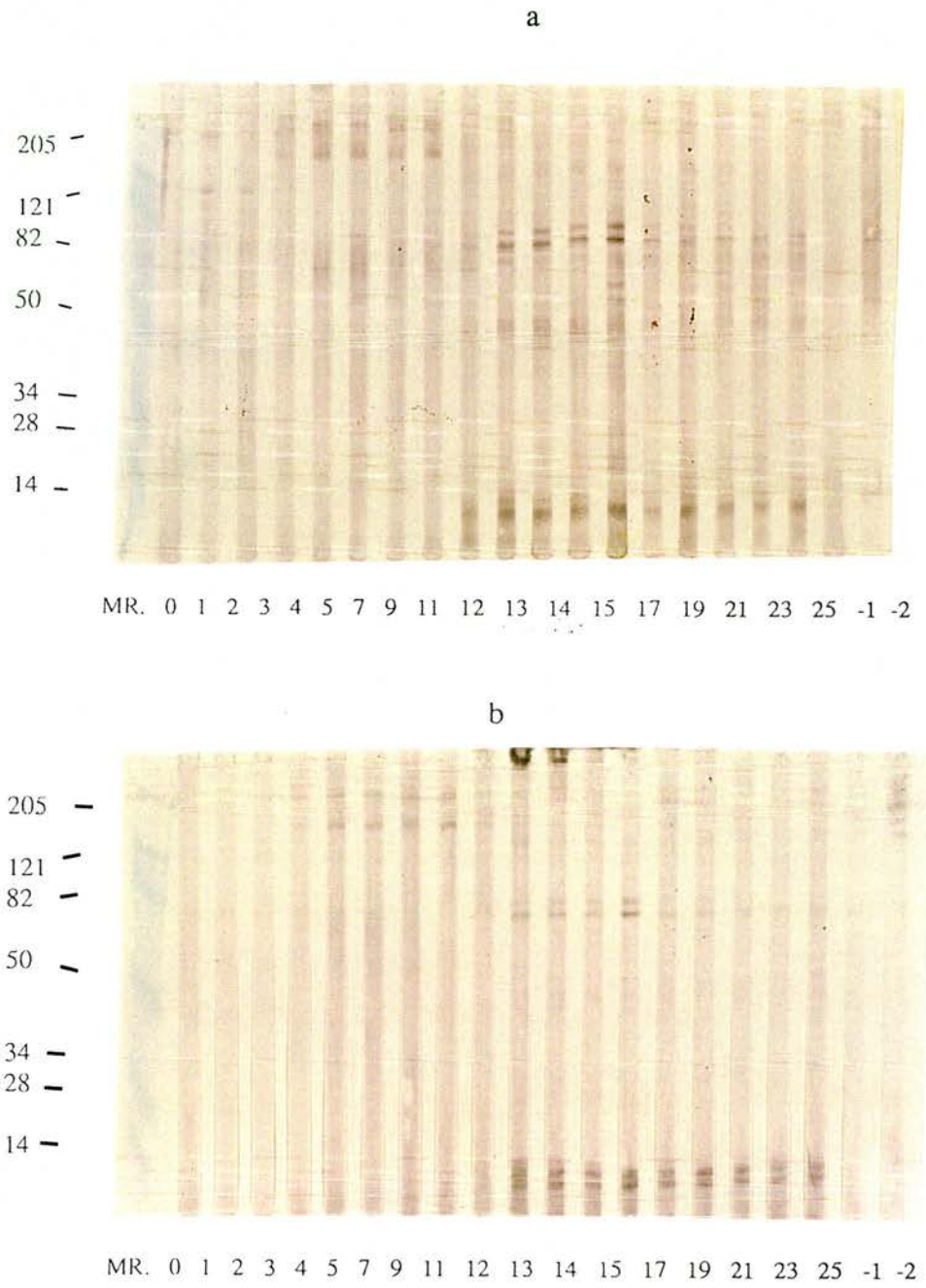
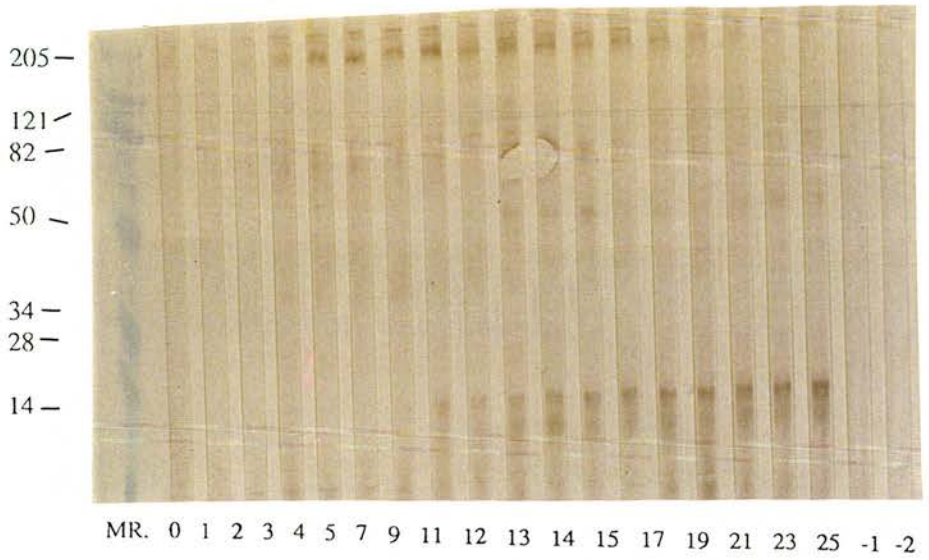


Figure 4.134 (a & b) The 24-48 hours Fg-E/S recognition by sera of *F. gigantica* infected calf 22 under reduced (a) and unreduced conditions (b) under reducing condition. The position of molecular weight markers (Mr) pre-bleed (-1) and diluent (-2) and the numbers at the bottom represent weeks post infection (wpi.) and the molecular weights are shown on the left hand side of the figures.

a



b

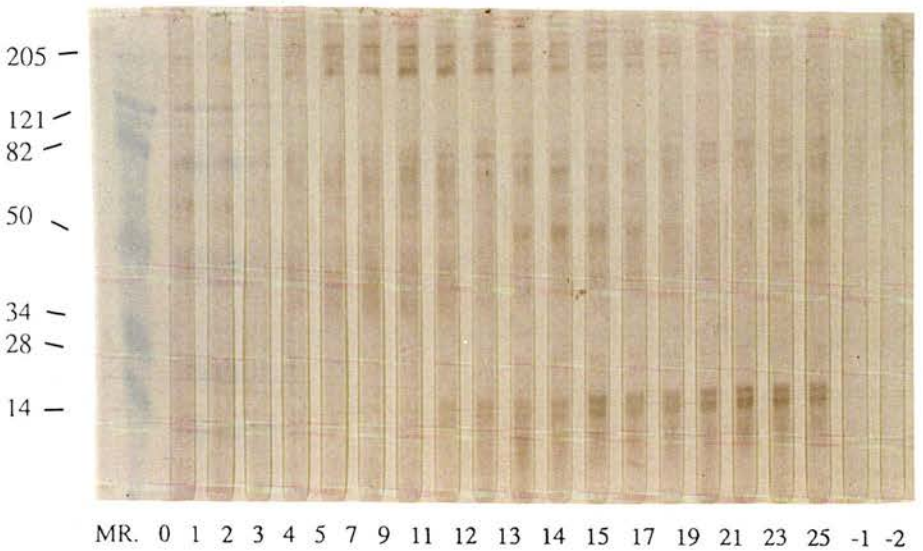
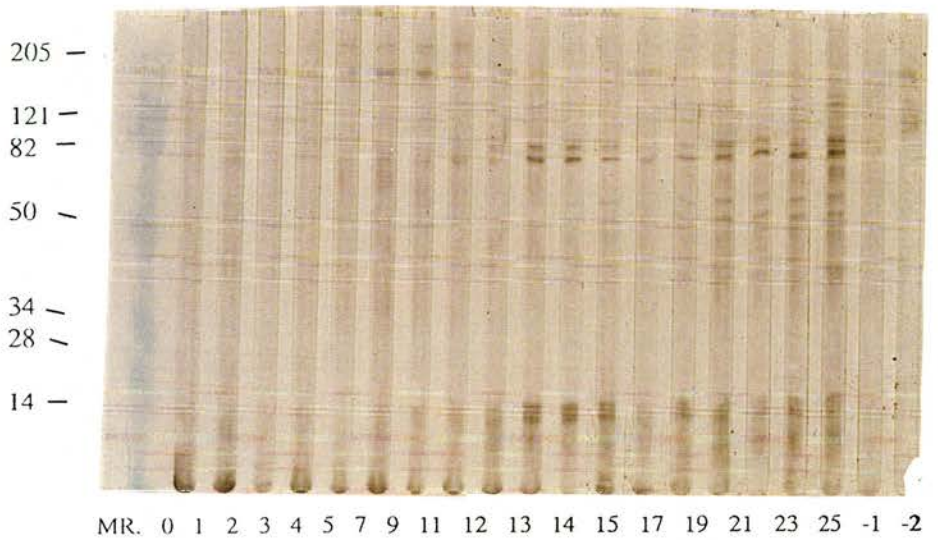


Figure 4.135 (a & b) The 24-48 hours Fg-E/S recognition by sera of *F. gigantica* infected calf 23 under reduced (a) and unreduced conditions (b) under reducing condition. The position of molecular weight markers (Mr) pre-bleed (-1) and diluent (-2) and the numbers at the bottom represent weeks post infection (wpi.) and the molecular weights are shown on the left hand side of the figures.

a



b

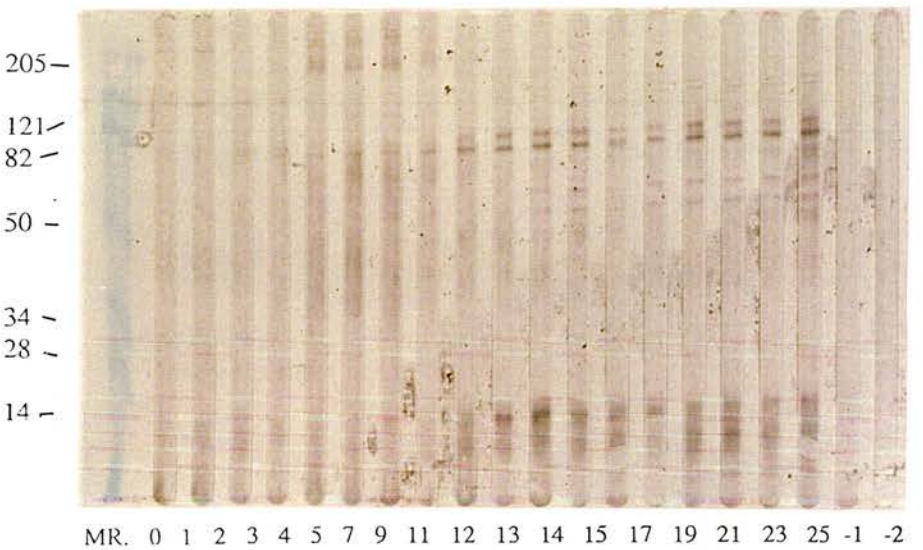


Figure 4.136 (a & b) The 24-48 hours Fg-E/S recognition by sera of *F. gigantica* infected calf 24 under reduced (a) and unreduced conditions (b) under reducing condition. The position of molecular weight markers (Mr) pre-bleed (-1) and diluent (-2) and the numbers at the bottom represent weeks post infection (wpi.) and the molecular weights are shown on the left hand side of the figures.

CHAPTER FIVE

5.0 DISCUSSION

The discussion is divided into four main sections, first the clinical status and disease pathogenesis of the *Fasciola spp* infected sheep and cattle used in this study, secondly their serum glucose and β-HOB levels, thirdly their serum and faecal antibody isotype responses to defined *Fasciola spp.* antigens and finally the general conclusions.

Fasciolosis is an economically important disease caused by digenic trematodes of the genus *Fasciola*. *F. hepatica* and *F. gigantica* are the two most important species responsible for fasciolosis. Both species are transmitted by snails of the family *Lymnaesidae*. *F. hepatica* prevails in temperate regions while *F. gigantica* is predominant in tropical zones (Over, 1982).

Fasciola spp. invasive larvae, or metacercariae, escape from their protective cyst in the duodenum or jejunum of the definitive host and penetrate the intestinal wall to reach the peritoneal cavity. Flukes specifically target the liver causing liver pathology and necrotic lesions, which result from the parasites' migration through the liver parenchyma. Further damage is caused when they enter the bile ducts causing haemorrhage (Malek, 1980). The second important factor is that flukes are haemophageous and infection results in anaemia.

Sinclair (1967) reviewed the pathogenesis of *Fasciola spp* in different definitive host species and concluded that there are broad similarities, but considerable species variation, in the pathology of fasciolosis. Thus *F. hepatica* and

F. gigantica, differ in their pathogenicity and related production loss with *F. gigantica* being more pathogenic and causing more production loss than *F. hepatica* (Hammond and Sewell, 1990; Mahato, 1993).

It is also established that there are host species differences in resistance to infection/reinfection with fasciolosis (Boray, 1969; Haroun and Hillyer, 1986; Bürger, 1992). Cattle, rats and goats develop strong resistance to challenge infection with *F. hepatica* and *F. gigantica* following primary infection or drug-abbreviated infection (Haroun and Hillyer, 1986) but accumulated evidence indicates that mice and the majority of sheep breeds do not develop such resistance (Boray, 1969). However, breed variation does occur as a small number of sheep breeds such as the Red Maasai sheep (Wamae, 1996) and Indonesian thin tailed sheep (Roberts, Estuningsil, Wijayanti, Wiedosari, Partoutomo and Spithill, 1997) are relatively resistant to *Fasciola spp.* infection/reinfection.

From the above information, it is clear that, in the study of cattle and sheep responses following infection either with *F. hepatica* or *F. gigantica*, we have a useful system in which to investigate the basis of species differences in susceptibility/resistance to parasite infection. Furthermore we can take advantage from employing two parasites which differ in the severity of the pathology they cause in definitive hosts.

The primary aim of this study was to examine whether the observed host species differences in resistance and susceptibility is related to species difference in the immune response following infection. This was done by examining the serum and faecal antibody isotype responses of experimentally infected sheep and cattle to

defined adult *Fasciola spp.* antigen (E/S, Cathepsin and GST) with the intention of relating these findings to what is currently known about helper T-cell responses.

The second aspect of the study was to examine the effects of fasciolosis on the serum glucose and β-HOB levels in sheep and cattle. In ruminants, the liver is the main store for glycogen and glycogen, which is a glucose reservoir, may constitute as much as 8% of the liver compared to 1% or less in the skeletal muscles (Breazile, 1971). The migrating flukes damage the liver resulting in severe liver pathology and death of hepatocytes, which may potentially reduce liver glycogen reservoirs (Berry and Dargie 1976; Ferre, Barrio, Gonzalez-Gallego and Rojo-Vazquez, 1994). A reduction in available liver glycogen will lead to a reduction in serum glucose levels and a corresponding increased mobilisation of free fatty acids, resulting in increased serum ketone bodies of which the β-HOB accounts for 80% (Doxey, 1983; Fraser, 1991; Sanchez, Alvareg and Lunusse, 1996). The aim therefore was to examine changes in glucose and β-HOB levels in infected sheep and cattle considering this in relation to the severity of infection. The rationale being that the potential interruption or disruption of carbohydrate metabolism may at least in part be responsible for serious impairment of the animal productivity and growth, a characteristic of fasciolosis.

5.1 PATHOGENESIS AND CLINICAL STATUS OF EXPERIMENTAL ANIMALS

This study mainly investigated the immunoglobulin isotype responses of sheep and cattle, chronically infected with *F. hepatica* and *F. gigantica,* to defined the fluke antigens (*F. hepatica* E/S products (Fh-E/S) or *F. gigantica* E/S products

(Fg-E/S), Fh-cathepsin and Fh-GST). Chronic infection was established because immunity is considered to play a potentially more important role in chronic fasciolosis than in acute infection. Acute fasciolosis is normally characterised by the early death of the animal through severe liver pathology caused by migrating flukes resulting in anaemia and haemorrhages caused by the flukes entering the bile ducts. In order to determine the severity of the disease in infected sheep and cattle, clinical findings, haematological, parasitologial, clinical biochemical and pathological parameters were recorded and analysed. All the infected animals developed a chronic fasciolosis with sheep showing a consistently more severe syndrome than cattle.

5.1.1 Clinical Findings

The direct relation in sheep between clinical signs and the level of metacercaria infection observed in this study is in agreement with Losos (1986) and Bürger (1992). For example sheep infected with 300-350 *F. hepatica* metacercariae displayed appetite depression and reduction in live weight gain, while the two sheep infected with 200 metacercariae showed no clinical signs, except for slight live weight gain reduction. However, the *F. hepatica* metacercariae infection doses used in all sheep were enough to cause chronic fasciolosis.

The infective doses of *F. gigantica* metacercariae in sheep were generally lower than that of *F. hepatica* but the overall clinical signs were more severe in *F. gigantica* infected sheep. The disease was so severe in sheep 13 infected with 100 *F. gigantica* metacercariae (45 flukes recovered) that it had to be culled 15 wpi. The

same problem lead to the premature culling of sheep 23 infected with 150 metacercariae (80 flukes recovered) and sheep 25 infected with 350 *F. gigantica* metacercariae (181 fluke recovered). The reasons for these differences in severity in clinical signs between *F. hepatica* and *F. gigantica* are considered to be due to the relative larger size of *F. gigantica* and the fact that *F. gigantica* spends a longer period in the liver parenchyma than *F. hepatica* (Ogunrinade, 1984a and Mahato, 1993).

The absence of clinical signs in some sheep and in all the cattle infections was considered due to the light infective dose which is in agreement with Hammond (1973), Wiedosari and Copeman (1990) and Radostits, Blood, and Gay, (1994). The lack of clinical signs in cattle was not surprising considering that the infective dose was low with the fluke burden per Kg live weight below 0.15. This is also in agreement with Bitakaramire and Bwangamoi (1969) who reported 500 *F. gigantica* metacercariae to cause clinical signs in calves.

In conclusion the sheep had a clear chronic fasciolosis with some sheep more severely affected but cattle displayed a chronic fasciolosis with few clinical signs.

5.1.2 Parasitology

There was significant difference between prepatent period of *F. hepatica* infected sheep and *F. gigantica* infected sheep ($t = 6.60$ $\rho<0.001$) suggesting that *F. hepatica* has a shorter prepatent period than *F. gigantica* in both sheep and cattle which is in agreement with Hammond (1970), Soulsby (1982) and Mahato (1994).

As reported by Mahato (1993) there was great variation in eggs counted in individual animals which had no apparent relationship with the worm burden.

5.1.3 Pathology

There were variable gross pathological changes in both sheep and cattle infected with both *Fasciola* spp. In general the lesions observed in this study are comparable to those reported by Hammond (1970), Ogunrinade (1984a), Ajanusi, Ogunsusi, Njoku, and Cryang, (1988) and Mahato (1993). As was expected the lesion were most severe in culled sheep and calves with single infection but culled earlier (11-16 wpi.) in the infection and those calves with challenge infection.

The lesions observed in sheep culled early in the experiments included enlarged livers with prominent haemorrhagic tracks and fibrous peritonitis. Some of the sheep had blood stained fluid in the peritoneal cavity. Ajanusi, Ogunsusi, Njoku and Cryang (1988) reported similar lesions in sheep infected with *F. gigantica* and Ogunrinade (1983) in cattle infected with *F. gigantica*. Mahato (1993) observed similar changes in water buffaloes. Only slight pathological lesions were observed in the majority of sheep livers, enlarged bile ducts being the most common.

Bitakaramire and Bwangamoi (1969) were able to show that the liver lesions are most severe during prepatent period of *F. gigantica* infection due to flukes migration. This explains why severe lesions were found only in animals culled in the early stages of infection in these studies. Calves showed severe calcification especially in those animals with challenge infection. This was in accordance with Bitakaramire and Bwangamoi (1969) and Jones and Hunt (1983) who reported that

calcification is part of the pathology of fasciolosis in cattle. Calcification was not observed in sheep as also reported by Mahato (1993).

One other prominent gross lesion was the presence of hepatic abscesses in sheep with more severe pathological changes. This is in agreement with O'Sullivan (1995) who after two years analysis of liver lesions from abattoirs in UK found that abscesses due to *F. hepatica* infections were common.

Histopathological lesions found in these experiments ranged from peripheral fibrosis, infiltration of mononuclear inflammatory cells in periportal areas, fibrosis and hyperplasia of the bile ducts. This in agreement with Mahato (1993). There were also eggs trapped in bile ductiles of livers of sheep 13 culled after 15 wpi. and in calf 23c challenged by 15 wpi. and culled 12 weeks post challenge.

5.1.4 Haematology.

The onset of anaemia was earlier in *F. hepatica* infected sheep than in those infected with *F. gigantica* and the anaemia was more pronounced in *F. hepatica* infected sheep with high infective dose per kg than in those infected with *F. gigantica*. The elevation in MCV, and lack of changes in the MCHC indicate that sheep infected with 200 *F. hepatica* metacercariae developed macrocytic normochromic anaemia while those infected with 300 *F. hepatica* metacercariae, had an elevated MCV and reduced MCHC suggesting a normocytic hypochronic anaemia. The other *F. hepatica* and *F. gigantica* infected sheep also displayed a macrocytic normochromic anaemia in agreement with Mahato (1993) who observed a macrocytic normochromic anaemia in his sheep fasciolosis experiments. The *F.*

gigantica infected sheep on the other hand had a predominantly normocytic normochromic anaemia in agreement with Sewell (1966), Hammond (1970) and Wiedosari and Copeman (1990). The absence of anaemia in the cattle experiments was considered to be due to the light infection.

Differential white blood cell counts agreed with Hammond's (1970) conclusions that the only clearly defined change occurring in fasciolosis in sheep and cattle is a consistently high eosinophilia with slight elevation in number of lymphocytes and neutrophils but monocytes and basophils fluctuate within a normal range.

5.1.5 Clinical biochemistry

There was an initial rise in serum total protein levels in all the infected sheep but less so in cattle. The rise was followed by a fall by 10-12 wpi. Hammond (1970) and Mahato (1993) findings agree with the above observations. Hammond (1970) was able to show that much of the initial increase in serum globulin fraction of the total protein concentration was due to γ-globulin fraction, he however also noticed an increase in the α- and β-fractions.

There appears to be a positive relationship between higher infective dose and severity of hypoproteinemia and hypoalbuminaemia in *F. hepatica* infected sheep. Hypoproteinaemia was more severe in sheep infected with *F. gigantica* which have lost more lost more protein than those infected with *F. hepatica* confirming that *F. gigantica* is more pathogenic than *F. gigantica* (Bürger, 1992).

Hypoalbuminaemia followed the same pattern as serum total protein except that there was no initial increase. Mahato, (1993) and Wamae (1996) found the same pattern in serum total protein and albumin in sheep and goats. Holmes, Dargie, MacLean and Mulligan (1968b) associated hypoproteinaemia in chronic ovine fasciolosis with hypercatabolism of albumin and globulin due to loss of protein into the bite ducts of the infected animals.

GLDH activities in both *F. hepatica* and *F. gigantica* infected sheep increased significantly in the third week of infection confirming the findings by Mahato (1993) and Wamae (1996). The increase in this serum enzyme activity was greater in infected sheep with high infective dose per kg live weight. The decline in serum GLDH by 11 wpi. in both *F. hepatica* and *F. gigantica* sheep is due to reduction of damage to the liver parenchyma because most flukes have reached the bile ducts and the parenchyma has started to regenerate (Wamae, 1996). The results agree with Hughes, Teacher and Harness (1974) who suggested that in *F. hepatica* infected goats, GLDH levels reach a peak at the period of maximum fluke activity in the liver parenchyma before they enter the bile ducts. In heavy infections the tissue damage is greater and the peak is very heavy activities the peak may be reached by 3 wpi.

Serum GLDH in cattle followed a similar pattern except the levels were lower possibly due to low infective doses used. In the *F. gigantica* infected calves however the decline in GLDH levels was later at 14 wpi. This may be explained by the fact that *F. gigantica* takes longer to migrate through the liver parenchyma than *F. hepatica*.

There appeared to be to no increase in GLDH after challenge infection in calves. Fernandez (1984) reported increased serum GLDH activities both after initial and challenge infection. This difference is perhaps because our challenge infective dose was only 100 *F. hepatica* metacercariae per calf while Fernandez (1984) used 1000 metacercariae per animal for both initial and challenge infection.

Other authors (Rowlands and Clampitt, 1979; Wamae, 1996) also found that in some animals there was a second small serum GLDH activity elevation. Wamae (1996) suggested that it could be due to late migration. This reasoning is similar to Rowlands and Clampitt (1979) who found that flukes were still in the migratory stage when the majority were in already in the bile ducts. It is also suggested that fibrosis of the liver parenchyma could lead to cell degeneration hence GLDH elevation in the serum (Wamae, 1996). Hughes, Teacher and Harness (1973) suggested after planting immature flukes in the biliary system of goats and recording elevated GLDH that this may be due to diffusion of toxic product or an auto-immune response.

The serum γ-GT activities in cattle on the other hand rose much later at 12 wpi. There was however, an earlier (4-5 wpi.) increase γ-GT serum activities in cattle which could be as a result of damage of small bile ducts as the flukes migrate to the main bile ducts as suggested by Bürger (1992).

Changes in serum GLDH and γ-GT levels during the course of experimentally induced infection with both *F. hepatica* and *F. gigantica* are in accordance with previous studies, which indicate that serum activities of liver enzymes are sensitive indicators of liver damage in sheep and cattle (Sykes, Coop

and Robinson, 1980; Mahato, 1993; Wamae, 1996; Ferre, Ortega-Mora and Rojo-Vazquez, 1997). Increased GLDH serum levels are related to migration of juvenile flukes through the liver parenchyma and are associated with inflammation and tissue destruction. On the other hand, the increase in serum γ-GT activities coincides with the entrance of adult flukes into the biliary system and is associated with hyperplastic cholangitis (Blood, 1994)

5.1.6 Glucose and β-hydroxybutyrate

The results in these experiments show reduction in glucose levels in infected sheep from as early as 3 wpi. This continued till the end of infection in both *F. hepatica* and *F. gigantica* infected sheep. There were no significant differences between calves infected with either fluke species and the uninfected control calves in serum glucose levels. The sheep results are in agreement with Ferre, Barrio, Gonzalez-Gallego and Rojo-Vazquez (1994) who reported decreased glucose values from 40 days post infection in lambs infected with *F. hepatica* while the controls showed no decrease. It is possible that the plasma glucose values were lower than normal due to the hepatic glycogenic pathways being depressed. Another factor to be considered for the reduction of glucose is the reduction of voluntary feed intake by the infected sheep. Kouider and Kolb (1994) also reported reduction of glucose in *F. hepatica* infected sheep prior to injecting them with ascorbic acid. However there is generally lack of research on the effect of fasciolosis on glyconeogenesis.

In ruminants food deprivation induces breakdown of body tissue and causes a shift in the main energy producing pathway, trichloroacetic acid cycle (TCA-cycle)

towards increased formation of ketone bodies (β-OHB and acetoacetate) as a result of insufficient supply of acetoacetate precursors (Wensvoort, Kyle, Rskov and Bourke, 1996). Thus in sheep and cattle infected with *Fasciola* spp, the migratory flukes damaging the hepatic glycogenic pathways and poor appetite may lead to increased production of ketone bodies. The poor nutrition status due to reduction in voluntary feed intake in sheep was corroborated by the high serum β-OHB in this study. This in agreement with Sánchez; Alvarez and Lanusse (1996) who reported that feed restriction in sheep does lead to increased β-OHB serum concentration. Wensvoort, Kyle, Rskov and Bourke, (1996) after starving sheep, cattle, camels and llama for 5 consecutive days with 6 weeks in between found that sheep and cattle recorded decreased serum glucose and increased serum β-OHB similar to the sheep results in this study.

5.2. SERUM ANTIBODY RESPONSES TO DEFINED *FASCIOLA* SPP ANTIGENS

This part of the study investigated the immunoglobulin isotype responses of sheep and cattle, chronically infected with *F. hepatica* and *F. gigantica*, to defined fluke antigens Fh-E/S, Fg-E/S, Fh-cathepsin and Fh-GST).

There was an early (2-3 wpi.) total Ig response to Fh-E/S and Fg-E/S, Fh-cathepsin and Fh-GST in both *F. hepatica* infected sheep and cattle. Although there was an early (2-3 wpi.) total Ig response to Fh-E/S and Fg-E/S, and Fh-GST by *F. gigantica* infected animals, there was a slight delay (7 wpi.) in the response to Fh-cathepsin. The pattern of the IgG_1 response of cattle and sheep to these defined fluke antigens was similar to that of total Ig. In fact the serum isotype response was

predominantly IgG_1. The IgM response to Fh-E/S and Fg-E/S, Fh-cathepsin and Fh-GST was early in both species. In cattle the IgG_2 and IgA responses to Fh-E/S and Fg-E/S were late (11 wpi. and 19 wpi. respectively) in contrast to sheep (2 wpi. for both isotypes). The isotype responses to individual antigens are discussed under respective antigens below.

There was no relationship between the serum antibody levels to Fh-E/S, Fh-E/S, Fh-Cathepsin and Fh-GST in indirect ELISA by individual animals and the infective doses (100, 150, 300 and 350 in sheep or 200, 450, 600 in cattle), fluke burden or pathological changes observed during *post-mortem*. This lack of influence in the primary infective dose in the antibody response has previously been reported in sheep with 250 and 500 metacercariae by Zimmerman, Farnsworth, Cerro and Wescott (1982) and more recently by Martínez, Martínez-Cruz, Martínez, Gutiérrez and Hernández (1996) in goats infected with 100 or 200 metacercariae. However, this apparent lack of difference may be related to the sizes of the infective doses, because Wyckoff and Bradley (1986) reported significant differences in cattle infected with 3000 and 30 metacercariae but not with 3000 and 300 or with 300 and 30.

The lack of relationship between antibody levels and the number of flukes recovered in this study agrees with Sandeman and Howell (1980). Dalton, McGonigle, Ralph and Andrew (1996) also reported lack of correlation between total Ig response, assessed by ELISA and immunoblot, and the levels of protection obtained in cattle vaccinated with Cathepsin-L1 protease and those vaccinated with Cathepsin-L1 protease Cathepsin-L1 protease and haemoglobin.

Cathepsin L1 protease is more immunogenic than Fh-GST however the current attempts to use it as a vaccine candidate for *Fasciola* infection in ruminants and humans (Dalton, McGonigle, Ralph and Andrew, 1996; Emery, 1996) appears to suggest that the molecule is of limited value as a vaccine candidate and that in future more molecules should be isolated from *Fasciola* spp. E/S products and examined for immunogenicity and protective properties.

5.2.1 *Fasciola* spp. E/S products

The measurements of antibodies against certain *Fasciola* spp. excretory/secretory products (E/S) in *Fasciola* spp infected animals may assist in the diagnosis of fasciolosis and also demonstrate an antigen recognition pattern that could be associated with immunity. Thus serving as a tool to investigate reasons why cattle are more resistant to primary (Ross, 1967; Boray, 1967, 1969) and to challenge infection (Doy, 1973; Fernandez, 1984) than sheep.

A number of studies have evaluated different antigens and test systems for the serodiagnosis of fasciolosis, and numerous attempts have been made to detect antibodies stimulated by *Fasciola* spp. infections (Nyanzunda, 1993; Wamae, 1996). The use of excretory/secretory parasite products (E/S) rather than somatic antigens afford a chance to study products actively produced by the living liver fluke. Somatic *F. hepatica* antigens were shown to be inferior to 24 hours E/S products in diagnosing experimental and natural infections (Sinclair and Wassall, 1988) using an ELISA method. Such products have been used in Western Blot analysis as well as ELISA for immunochemical analysis and diagnosis studies. Martinez-Moreno, Jemenez,

Martinez-Cruz, Martinez-Moreno, Beccerra and Hernandes (1997) used *F. hepatica* E/S products as antigen in an Enzyme-Linked Immunosorbent Assay (ELISA) to asses drug efficacy in goats with experimental and natural infections.

This study has clearly demonstrated that *F. hepatica* and *F. gigantica* infected sheep and calves develop clear total Ig responses to *F. hepatica* E/S (Fh-E/S) and *F. gigantica* E/S (Fg-E/S) starting from 2 weeks post infection and reaching high levels by 8 wpi. in sheep and 3-7 wpi. in cattle. This early response to adult *Fasciola* spp. E/S antigen suggests an early exposure to the antigen presumably through the cross-reacting E/S products of juvenile flukes. This does suggest that juvenile flukes secrete enough antigenic E/S products to provoke a detectable antibody response. These results are in agreement with those obtained by Wamae (1996), who reported specific total Ig responses to Fg-E/S by 2 wpi in sheep and cattle with primary *F. gigantica* infection. Also with Santiago and Hillyer (1988) who, assessing the reactivity of sera from ruminants with *F. hepatica* using somatic and E/S antigens by ELISA and Western Blot, found that major antigenic E/S polypeptides of 23-28 kDa were detected by 4 weeks post infection. In addition Sinclair and Wassall (1988) found that there was greater sensitivity and consistency in antigens prepared from metabolic and secretory products compared with somatic antigens.

Few sequential immunoblot, Western Blot, analyses have been carried out to monitor the total Ig response to Fh-E/S and Fg-E/S in the course of infection in both sheep and cattle infected with *F. hepatica* and *F. gigantica*. The Western Blot results in calves showed that all *F. hepatica* infected calves sera first recognised a 142 kDa doublet by 7 wpi. and a 134 kDa molecule by all *F. gigantica* infected calves. A new

prominent 60 kDa molecule in both groups of cattle replaced the higher one after patency. Other prominent molecules recognised in the course of infection was a 14 kDa doublet in both *F. hepatica* and *F. gigantica* infections. There was a clear consistent shift in antigen recognition by cattle from higher (134 kDa for *F. gigantica* infection and 142 kDa for *F. hepatica* infection) to lower (60 kDa for both parasite species) affirming the reports by Nyanzunda (1993) and Wamae (1996), who examined *F. gigantica* infected cattle, but there was no clear shift in antigenic recognition by sheep sera.

In *F. hepatica* infections in sheep Western Blot analysis identified 14, 54, 79 and 134 kDa proteins whereas *F. gigantica* infected sheep sera recognised products of 14, 88 and 152 kDa. However comparison of molecular weights of immunoreactive proteins with the published data was difficult due to differences in methods used to calculate these weights. These results are close to those reported by Chauvin, Bouvet and Boulard (1995) who observed in sheep infected with *F. hepatica* 12 major antigenic proteins ranging from 9 to 156 kDa with those of 20, 24, 51 and 69 kDa described as specific for *F. hepatica*.

There were differences in recognition pattern between sheep and cattle as well as within the same host species. These variations in recognition pattern of antigenic molecules by different host animals during the course of infection was also reported by Marrero, Santiago and Hillyer (1988) and Wamae (1996).

The present work clearly shows that there is a shift in antigen recognition in cattle at or after the time of patency (i.e. eggs in faeces) but not in sheep. The consequences of this difference is open to speculation but it could be that the lower

Mwt antigens are involved in resistance in cattle fasciolosis which would imply a protective role for the 60 kDa antigen. The role of a 14 kDa antigen is unclear considering that it is detected by both sheep, the host considered susceptible, and cattle the resistant host.

Antibody isotype analysis indicated that in infected sheep the antibody response to Fh-E/S and Fg-E/S was predominated by IgG_1 while IgM, IgG_2 and IgA responses could also be detected. The results in cattle also indicated that the response was predominantly IgG_1 from 3 wpi. however IgG_2 response was equally strong but late (19 wpi.) in *F. hepatica* infected cattle and there were clear detected IgA responses in both *F. hepatica* and *F. gigantica* cattle. This is in agreement with Movsesijan, Jovanovic, Aalund and Nansen (1975) who, after employing the indirect fluorescence antibody technique in *F. hepatica* infected sheep found that IgG_1 response was dominant to *F. hepatica* digestive tract antigens. Clery, Torgerson and Mulcahy, (1996) working with cattle also reported a predominantly IgG_1 response to adult *F. hepatica* somatic antigen in *F. hepatica* infection. The IgG_1 subclass dominance in ruminant fasciolosis has also been detected by Poitou, Baeza and Boulard (1993) IgM, IgG_1 and IgG_2 antibody isotype response to adult fluke 0-24 hour E/S antigen in rats with primary *F. hepatica* infection. This isotype, IgG_1, dominance is also reported in other helminth infections. For example McKeand, Duncan, Urquhart and Kennedy (1996) reported an initial IgM response preceded with IgG_1 and IgG_2 in calves infected with or vaccinated against *Dictyocaulus viviparaurus* to larval and adult *D. viviparaurus* surface antigen with IgG_1 clearly the dominant isotype.

Sheep exhibited an early but low IgA and IgG_2 response to Fh-E/S and Fg-E/S, Doucil, Green and Risdon (1994) also reported these isotype responses in sheep infected with *Trichostrongylus colubriformis* and treated with Dexamethazone.

The comparison of the isotype responses between infected sheep and infected cattle shows that cattle developed early total Ig, IgG_1, IgM but late response IgG_2 and IgA, while in the infected sheep all the isotypes developed earlier. The sheep IgG_2 and IgA responses were generally lower than in cattle and lasted for a shorter period (ca. 3-4 weeks). In *F. hepatica* infected cattle the IgG_2 response, though late, was stronger than in *F. gigantica* infected cattle.

The differences in responses between host species is difficult to explain considering that not all immunogenic components of *Fasciola* spp. E/S have been isolated and their influence on individual immune response to *Fasciola* spp. infection analysed.

This earlier detection of an antibody detection of an antibody response indicates a similar antigenic pattern between juvenile and adult flukes with respect to Fh-E/S and Fg-E/S. Juvenile flukes share antigens with both metacercariae and adults (Sandeman and Howell, 1980).

After challenge, the two *F. hepatica* infected and challenged calves (15c and 23c) showed no increased IgM, IgG_2 or IgA to E/S however in calf 15c total Ig and IgG_1 response increased slightly. This increase suggests that juvenile flukes from challenge infection releases sufficient antigenic materials to cause this increase in antibody response. The increased levels of ELISA values after a second infection is

in agreement with Fernandez (1984), who reported increased antibody levels after challenge infection as compared to the primary infection in either bullocks or in rats.

5.2.2 Cathepsin-L protease from adult *F. hepatica*

The antigenicity of Fh-cathepsin was confirmed in that the sera of all infected sheep and cattle reacted against this enzyme, and in general, the antibody profile revealed an earlier detection of total antibody responses (total Ig) from two to four weeks post infection. These results are in agreement to those obtained by Levieux, Levieux, Mage and Venien (1992) in sera from calves using the specific antigen f2 of *F. hepatica* in a passive haemagglutination test. The results obtained here reveal that the total Ig and isotype IgG_1 responses began earlier in the infection, suggest that Fh-cathepsin a proteolytic enzyme is also produced by juvenile flukes. Juvenile flukes share antigens with both metacercariae and adults (Sandeman and Howell, 1980). This corresponds to the findings of previous studies (Dalton and Heffernan, 1989) which revealed that proteases are functional even in invasive flukes i.e. newly exysted juveniles, therefore proteases are secreted by all stages of liver flukes that exist in the mammalian host (Carmona, Dowd, Smith and Dalton, 1993).

This study has clearly demonstrated that *F. hepatica* and *F. gigantica* infected sheep and cattle developed clear total Ig responses to Cathepsin-L1 protease of adult *F. hepatica* starting from 2 wpi. and reaching the peak from 8 to 22 in sheep and 3 wpi. to end of experiment in *F. hepatica* infected cattle and 8 wpi. in *F. gigantica* infected cattle. The high levels of total Ig responses are in agreement with findings in

humans and other animals with *Schistosoma mansoni* infection (Chappell, Dresden, Gryseels and Deelder, 1990).

Antibody isotype analysis in *F. hepatica* and *F. gigantica* infected sheep indicated that there was predominantly IgG_1 while IgG_2 and IgA responses could be detected in *F. hepatica* infection to some extent. IgG_2 and IgA responses were less marked in the *F. gigantica* infected sheep. In *F. hepatica* and *F. gigantica* infected cattle there was predominately an IgG_1 response but unlike in sheep, late IgG_2 and IgA responses were clearly detected. IgM, though variable, in both hosts was more pronounced in cattle. However, there was late (ca. 8 wpi.) total Ig and IgG_1 response to Fh-cathepsin by *F. gigantica* infected cattle. This may suggest a partial cross-reactivity between *F. hepatica* and *F. gigantica*.

These results can be compared with those reported in the related trematode like *Schistosoma* spp. since little similar work has been done in *Fasciola spp.* The antibody responses to purified *Schistosoma spp.* protease have been monitored in different hosts, including man. The antibody isotypes results reported in this study, especially in cattle, are similar to the findings by Bout, Rousseaux, Carlier and Capron (1980) who found early IgG_1 and IgM antibodies responses and late IgA (80 days post infection) during *Schistosoma mansoni* murine infection. Chappell and Dresden (1988) also reported that response to a SMw32, purified protease, primarily involves IgM and IgG_1 antibodies. Later, sera from *S. mansoni* infected individuals were tested for IgM and IgG cysteine protease antibodies (Chappell, Dresden, Gryseels and Deelder, 1990).

Mountford, Fisher and Wilson (1994) reported that *S. mansoni* cercariae infected mice developed a serum IgG_1 response to soluble antigens from different developmental parasite stages, including adult worms, between weeks five and seven, whereas IgG_2 responses were lower.

Kamata, Yamada, Uchikawa, Matsuda and Arizono (1995), examined the allergenicity of a 16 kDa purified cysteine protease of the nematode, *Nippostrongylus brasiliensis*, which stimulated high levels of IgG_1 and lower levels of IgE and negligible levels of IgG_2 in rats. Although anti-cysteine protease IgE was not examined in the present work, the other isotype responses were very similar to this experiment.

The total Ig and IgG_1 response in homologous infection was earlier, 2 wpi. for sheep and 4 wpi. in cattle than in heterologous infection, 8 wpi. in sheep and +7 wpi. in cattle. Therefore, Fh-cathepsin activity against heterologous infection (i.e. *F. gigantica*) needs to be ascertained. Although the sera from sheep separately infected with *F. hepatica* and *F. gigantica* recognised the Fh-cathepsin antigen, the level of antibodies reacting with the purified enzyme was higher in *F. hepatica* infected animals.

5.2.3 Glutathione S-Transferase of adult *F. hepatica* (Fh-GST)

This study has clearly demonstrated that *F. hepatica* and *F. gigantica* infected sheep and cattle developed clear total Ig responses to Fh-GST of adult *F. hepatica* starting from 2 wpi. Antibody isotype analysis indicated that the response was predominately IgG_1 and IgM while IgG_2 and IgA response was either non existent in

some sheep or cattle or just barely detectable. IgG_2 and IgA responses were more marked in the cattle. All isotype responses were variable especially in sheep.

The total Ig responses to adult Fh-GST began early in the infection suggesting that, as with Fh-cathepsin, Fh-GST could also be produced by juvenile flukes and that they are capable of producing enough Fh-GST to provoke detectable humoral immuneresponse. This is in agreement with previous studies by Hagan and Gryseels (1994) who reported that *Schistosoma mansoni* GST (Sm28GST) are found in different life cycle stages. Bal and Das (1996) on the other hand suggested that in *S. mansoni* GSTs is most abundant on the surface of the adult fluke and in the eggs. However there is no evidence to suggest that this abandance in GST in adult and eggs has any influence on isotype pattern in ruminants.

The antigenicity of Fh-GST was confirmed in that the sera of all infected animals reacted against this enzyme, and in general, the antibody profile revealed an earlier detection of total antibody responses (total Ig) from two to four weeks post infection. These results are in agreement to those obtained by Hillyer, Soler de Galanes and Battisti (1992) who were able to detect anti-Fh-GST in sera from sheep using the specific antigen Fh-GST of adult *F. hepatica* in an ELISA.

However the antibody responses to GST were poorer than that to *Fasciola* Fh-E/S, Fg-E/S and Fh-cathepsin. Unlike the results to Fh-cathepsin there was no sharp antibody response by infected sheep or cattle and there were variations in response in all the isotypes analysed. This might explain why Hillyer, Soler de Galanes and Battisti (1992) could not record any response in cattle and rats infected with *F. hepatica*. These authors found that sheep and rabbits infected with *F. hepatica*

clearly developed anti-Fh-GST antibodies but in contrast cattle and rats infected with *F. hepatica* did not respond to Fh-GST. They further concluded that one can divide the four host species into Fh-GST responders (sheep and rabbits) and non responders (cattle and rats). The results in this study clearly are different. The Hillyer, Soler de Galanes and Battisti (1992) findings especially those in cattle and rats may be due to many factors including the assay itself. These results also differ to those reported by Sexton, Milner, Panaccio, Weddington, Wijfells, Chandler, Thomson, Wilson, Spithill, Mitchell and Campbell (1990) who could not demonstrate antibody response to Fh-GST in sheep infected with *F. hepatica* by 6 wpi., however they did notice a slight response by 12 wpi.

The anti- Fh-GST isotype response was mainly limited to IgG_1 and IgM in both infected sheep and cattle but cattle showed slight IgG_2 and IgA response. The GST used as antigen in these studies was isolated from adult *F. hepatica*, there is clear evidence therefore of cross-reactivity with *F. gigantica*. The anti-Fh-GST antibody responses were observed in both *F. hepatica* and *F. gigantica* infected sheep and cattle and this included all the isotypes tested in this study, i.e. IgG_1, IgM, IgG_2 and IgA.

These results can be compared with those reported in the related trematode *Schistosoma spp.* as similar work in *Fasciola* has not been well documented. The antibody responses to purified *Schistosoma* GST has been monitored in different hosts, including man. The antibody isotypes were also reported by Auriault, Gras-Masse and Pierce (1990) who found increases in IgE, IgA and IgG_4 antibodies during schistosomiasis in humans. Generally there are very few reports concerning IgG_1

and IgG_2 responses to parasite GST even in well researched helminths like *Schistosoma spp.* or *N. brasiliensis*.

The present results are similar with those of Bal and Das (1996), who reported responses in human infected with *Wuchereria bancrofti* to GST. These authors reported that GST illicits predominantly a IgG_1 and IgM response and low levels of IgA. They also recorded high IgE responses in humans from endemic areas. As in the cattle studies presented here, these authors found that IgM isotype responses were higher than total Ig and IgG1 earlier in infection but IgG1 became the dominant isotype in the late stages of infection.

In conclusion, these results indicate that the Fh-GST promotes a dominant IgG_1 and IgM isotype response with the IgG_1 response being more persistent than the IgM response.

The response in cattle was gradual and total Ig was lower than IgG_1 and IgM. Helminth GST are known to conjugate with other endogenous substances such as lipid hydroperoxides and are thought to have a role in protecting the parasite against radicals produced by the host (Brophy and Barrett, 1990). Thus rats are known to produce 30 times more radicals per animal than mice in response to *F. hepatica* (Smith, Ovington and Boray, 1992). Rats and cattle are considered as more resistant to *Fasciola* infection (Boray, 1969; Malek, 1980; Haroun and Hillyer, 1986) and this might help explain the initial poor response to Fh-GST in cattle (resistant hosts produce more radicals) as compared to sheep (susceptible host produce less radicals).

This is the first comparative study of serum isotype responses to *F. hepatica* in sheep and cattle infected with either *F. hepatica* or *F. gigantica* and more

experiments need to be done in order to understand the influence of GST on protection and its contribution to host resistance against fasciolosis. The relationship of this isoenzyme to hydrogen radicals produced by the hosts need to be established. In order to understand the individual Ig isotype response and T-cell subset response experiments examining the cellular responses are necessary.

5.3 COPRO-ANTIBODIES TO *FASCIOLA* SPP. E/S PRODUCTS, CATHEPSIN-L1 CYSTEINE PROTEASE AND FH-GST

Since the parasite invades the host via the gut the response at gut level is of importance in protection. The study of copro-antibody afforded a non invasive way of looking this.

There was no faecal antibody response detected in cattle to any of the three defined antigens. This might have been due either to the light infections observed in cattle or to the larger volume of faecal material produced by cattle (i.e. dilution). There was an early (2 wpi.) faecal total Ig response to Fh-E/S and Fg-E/S, Fh-cathepsin and Fh-GST in *F. hepatica* and *F. gigantica* detected in infected sheep. These results are in agreement to those obtained by Wedrychowicz, Turner, Pfister, Holmes. and Armour, (1984) in antibody detection in faecal from Scottish blackface sheep. The faecal antibody responses were in this study similar to the different antigens by either *F. hepatica* or *F. gigantica* infected sheep.

The isotype response was mainly IgA while a slight IgG_2 response could be detected in *F. hepatica* infected sheep. The faecal IgA antibody isotypes reported here are in accord with Ellis, Gregory, Turnor, Kalkhoven, and Wroth (1993) who

reported significant increases ($p<0.02$) of *F. hepatica* IgA reactors in infected sheep compared to uninfected sheep.

The total Ig and IgA responses to all three defined antigens in *F. hepatica* infected sheep was biphasic, in *F. gigantica* infection however the phases were less defined. This pattern may be due to the fact that the IgA stimulation after oral infection reduces as the parasites migrate through the liver parenchyma then the adult fluke antigens passing with bile in the digestive tract reinforces the antibody stimulation leading to the second peak IgA. Thus the first phase (about 2-10 wpi.) is consistent with a response to juvenile flukes antigens after oral infection and the second peak (13-17 wpi.) a response to antigens released by adult flukes in the bile duct.

Wedrychowicz, Maclean, and Holmes (1985) reported a seven fold increase of IgA, a three to six fold IgG_1 and a fifty fold increase in IgM in primary *Nippostrongylus brasiliensis* infected rats. As in our findings the authors above reported an early total Ig and IgA. Ogunrinade (1983) reported an IgG_1 antibody in bile which correlated with circulating (sera) IgG_1 and suggested that IgG_1 in the bile is as a result of a leakage of serum IgG_1.

There was no correlation in IgA response to faecal egg counts in these studies. Gill, Gray, Watson and Husband (1993) on the other hand reported a negative correlation between IgA and faecal egg counts and suggested that IgA may be involved in host resistance.

5.4. GENERAL DISCUSSION AND CONCLUSIONS

The differences in susceptibility/resistance between sheep and cattle to *Fasciola spp.* infection is well documented (Haroun and Hillyer, 1986), but little is known about the underlying protective mechanisms. A comparison of serum isotype immune responses of these definitive hosts to primary *Fasciola spp.* infection will help our understanding of protective immunity in resistant (cattle) as compared to susceptible (sheep) hosts. The main objective of this study was to investigate the faecal and serum immunoglobulin isotype (IgG_1, IgG_2, IgM and IgA) responses of sheep and cattle chronically infected with either *F. hepatica* or *F. gigantica*, to defined fluke antigens (*F. hepatica* E/S products (Fh-E/S), *F. gigantica* E/S products (Fg-E/S), Fh-cathepsin and Fh-GST).

The complexity of these trematode parasites is reflected in the variety of molecules in their E/S compartment, which directly interacts with the host. Fluke antigenic E/S products are of particular interest since the function of some of these components is to either stimulate or modulate the host immune responses.. Two important E/S components were selected for detailed study, firstly the secreted enzyme Fh-cathepsin which has a MWt of 27 kDa. It is considered to have a functional role in parasite evasion of the host immune response, through cleavage of host immunoglobulin. The second enzyme, Fh-GST is also an E/S product and is of 27.8-29 kDa MWt. It is involved in the detoxification of exogenous (xenobiotic) and endogenous derived toxic compounds. Both enzymes are of interest because they have also been considered as vaccine candidates against fasciolosis (Hillyer, Soler de

Galanes and Battisti, 1992; Dalton, McGonigle, Ralph and Andrew, 1996; Emery, 1996).

The first objective of this study was to determine whether there are host species differences in the serum and faecal isotypes (IgG_1, IgG_2, IgM and IgA) responses to these defined antigens which may be related to host susceptibility/resistance and whether any observed differences are associated with the degree of pathogenicity. The possibility of host differences in the antigen (E/S) recognition patterns was examined by Western Blotting.

The results clearly demonstrated that *Fasciola spp.* infection provokes a dominant and rapid IgG_1 serum response to E/S, Fh-cathepsin and Fh-GST in both sheep and cattle. However, the response is more marked, and of longer duration, in cattle (resistant) compared to sheep (susceptible). Cattle exhibited a late IgG_2 serum antibody response to the defined antigen while sheep did not. Again unlike sheep, infected cattle produced a strong IgG_1 and IgM response to Fh-GST which rose throughout the infection. The implication of these findings are discussed in detail (see sections 5.2) however, the indications are that the persistence of a IgG_1 isotype response with a late IgG_2 isotype response to the parasite, as seen in cattle, may be associated with resistance.

Adult Fh-E/S, Fg-E/S and Fh-cathepsin evoked a stronger antibody response than adult Fh-GST especially in cattle which may reflect the immunogenicity of the two enzymes in sheep and cattle. The fact that antibody responses to adult Fh-cathepsin and Fh-GST were also detected in *F. gigantica* sheep and cattle indicates species cross-reactivity. Antigenic crossreactivity obviously exists between

components of juvenile and adult flukes as indicated by the early detection of antibody responses to adult fluke antigens. However, the relatively late detection of serum IgG and IgG_1 antibody to Fh-cathepsin in *F. gigantica* infected sheep and cattle may indicate that this cross-reactivity is only partial. Further isotype analysis of the antibody responses to *Fasciola spp.* infection involving a larger number of defined antigens, particularly those from juvenile and adult E/S products is clearly required.

In faeces, IgA was the main isotype antibody directed against all three defined antigens in both *F. hepatica* and *F. gigantica* infected sheep and this response was biphasic. The lack of detectable anti-parasite IgA in cattle faeces may be due to light infection or assay in sensitivity rather than absence of antibody isotype. It is therefore considered inappropriate at this point to draw a final conclusion on the role of intestinal IgA.

Unfortunately it was not possible to include an analysis of the IgE response of sheep and cattle to *Fasciola spp.* infection. This is an obvious omission, but we have since developed an assay to detect anti-parasite IgE in the sheep system and are in the process of developing another assay for cattle (Harrison, Personal Communication).

In the present studies an eosinophilia was first detected at 2 wpi. and dropped by 17 wpi. in sheep infections but remained high for up to 30 wpi in infected calves, especially those calves infected with *F. gigantica.* The levels of eosinophilia, particularly in cattle infected with *F. hepatica,* was generally lower than sheep. The main reason for this was considered to be that sheep were given a higher infective dose per kg live-weight than cattle. The persistence of an eosinophilia as found in

cattle may be associated with resistance as is thought to be the case with other helminths. For example, in *Schistosoma mansoni* infection, eosinophils are important effector cells, killing invading juvenile parasites by antibody-dependant cell-mediated cytotoxicity (Butterworth, Sturrock and Houba, 1975). It is known that the eosinophil adherence to *Fasciola* spp. is antibody mediated (Butterworth, 1984). In schistosomiasis, *in vitro* experiments demonstrated that eosinophils are involved in antibody dependant cytotoxicity in association with IgE (Capron, Dessaint, Caporn, Ouma and Butterworth, 1987). In rat fasciolosis, eosinophilia was also observed to be correlated with an increase in IgE (Pfister, Turner, Currie, Hall and Jarret, 1983; Poitou, Baeza and Boulard, 1993) .

Western Blotting analysis using 48 hours adult fluke E/S products, clearly showed that there is a shift in the antigen recognition pattern just after the time of patency (i.e. eggs in faeces) in cattle but not in sheep. There was a clear consistent shift in antigen recognition by cattle from higher (134 kDa MWt for *F. gigantica* infection and 142 kDa MWt for *F. hepatica* infection) to lower molecule (60 kDa MWt for both parasite species). The 60 kDa MWt doublet recognised by *F. hepatica* and *F. gigantica* infected cattle after patency may stimulate a protective resistance. However, due to the very small sample size examined in this investigation, these results require confirmation. Also of interest would be a Western Blotting analysis of the isotype responses of sheep and cattle to the 60 kDa MWt doublet discussed above, the three antigens used in this study and other antigens particularly by those from juvenile.

In mice and man, normal helper T-cells are divided into two subgroups with distinct functions, they are designated Th1-cells and Th2-cells. These subpopulations respond in different ways to cytokines and produce different interleukins in response to antigen stimulation. Thus in mice Th1-cells secrete characteristically cytokines IFN-γ and IL-2 while Th2-cells are characterised by the secretion of IL-4, IL-5, IL-6 and IL-10 (Finkelman, Pearce, Urban, and Sher, 1991; Tizard, 1995). This division of T-cells into Th1-cells and Th2-cells was first done in mice and was found to be applicable in human immune system, although with important differences such as the ability of both Th1-cells and Th2-cells in human to express IL-10 (Delprete, Decarli, Almerigogna, Giudizi, Biagiotti and Romagna, 1993). There is little information about Th1-cells and Th2-cells response and their characteristic cytokines in sheep and cattle, however, Brown, Hash and Rice-Ficht (1993) reported expression of IL-10 by Tho, Th1-cells and Th2-cells in cattle. In helminth infected mice, Th1 immune response induces IFN-γ hence IgG_{2a} secretion, while Th2-cells immune response induces IL-4 which stimulates IgE, IgG_1 and IgG_3 secretion (Finkelman, Pearce, Urban, and Sher, 1991; Tizard, 1995).

If similar events occurred in *Fasciola spp.* infected sheep and cattle, the dominance of a serum IgG_1 response and faecal IgA response may be explained. Results in the present study show that serum IgG_1 was the dominant isotype response to all three antigens suggesting that these antigens may preferentially stimulate a Th2 T-cell subset response. The late IgG_2 response to Fh-E/S, Fg-E/S and Fh-cathepsin in cattle may indicate delayed Th1 T-cell subset stimulation. In a limited study Brown, Davis, Dobbelaere and Rice-Ficht (1994) reported that T-cells clones derived

from cattle infected with *F. hepatica* produced cytokine profiles reflective of a Th2-cell, involvement i.e. similar to the results reported in mice and man that helminth antigens can induce Th2-cells.

Allen and Maizel (1997) concluded that the available evidence suggests that in many infectious diseases, both T helper cell (Th1- and Th2-type) are involved especially in the avoidance of severe immunopathology. Thus differences in antibody isotype responses of sheep and cattle to fasciolosis may play an important role in the "self cure" phenomenon and resistance against challenge infection observed in *Fasciola spp.* infection in cattle but not sheep. Helminth infection is universally associated with high levels of IgE, eosinophilia and a rise in mast cell numbers (Malek, 1980). The Th2-cell class of helper T-cells is known to be involved in eosinophil differentiation in man and mouse (Spry, Kay and Gleich, 1992) as they produce IL-3, IL-4 and IL-5 (Mosman, 1991). IL-5 is known to be a growth factor for eosinophils in mice, as injection of this cytokine alone stimulates eosinophilia (Coffman, Seymour, Hudak, Jackson and Rennik, 1989). The demonstration that eosinophils and IgE can kill helminth parasites *in vitro* has lead to the wide belief that Th2-dependent responses are primarily responsible for the destruction of large extracellular parasites (Tizard, 1992).

Thus if the eosinophil stimulation mechanism in ruminants is similar to that observed in mice, the persistence of IgG_1 response with the implied Th2-cell driven eosinophilia and IgE involvement may be of great importance to the resistance observed in cattle as well as the late development of antibodies of IgG_2 subclass with the implied Th1-cell involvement. These observations plus the fact that cattle

display a switch in their antigen recognition profile following patency may be another critical factor in the IgG1/IgG2 profiles. This latter point further emphasises the need for more detailed study in the antibody isotype responses to defined fluke antigens importantly the 60 kDa Mwt doublet. Further cellular studies are therefore recommended in order to help define the mechanism of protection in cattle.

The second part of the study dealt with serum glucose and β-HOB levels in both sheep and cattle. Migrating flukes damage the liver (Mahato, 1993), this may potentially reducing liver glycogen reservoirs. This could result in a reduction in glucose levels and an associated increased mobilisation of free fatty acids detectable through increased levels of serum ketone bodies. In ruminants β-HOB accounts for 80% of all ketone bodies (Doxey, 1983; Fraser, 1991; Sanchez, Alvareg and Lunusse, 1996). Analysis of serum glucose and β-HOB levels in resistant and susceptible hosts would therefore help the understanding of possible changes in carbohydrate metabolism (i.e. energy source) in fasciolosis. The reduction in serum glucose and increase in β-HOB levels observed in *Fasciola spp.* infected ruminants in this study supports this hypothesis. While such direct comparative studies have not previously been conducted, the available evidence on the changes in intermediate carbohydrate metabolism in fasciolosis supports our findings (Berry and Dargie 1976; Ferre, Barrio, Gonzalez-Gallego and Rojo-Vazquez, 1994; Sanchez, Alvarez and Lunusse, 1996).

In further support of this contention was the observation that changes in intermediate carbohydrate metabolism tended to be more pronounced in sheep (the

susceptible host) than in cattle and further they were more pronounced in *F. gigantica* infection (the more pathogenic of the parasites).

These changes in intermediate carbohydrate metabolism could be expected to be of importance to animal productivity particularly in young growing animals. Thus the dietary supplementation of carbohydrate intake may be expected to ameliorate the effects of fasciolosis. However, as this is one of the first studies of its kind, further work is required in order to verify these suggestions.

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APPENDIX 3.1

1 Preparations of Solutions and reagents.

Solution A

Algal culture
Potassium nitrate
Potassium dehydrogen orthophosphate
Sodium chloride
Sodium carbonate
Ferrous sulphate

Solution B

Ammonium vanadate
Boric acid
Cobalt nitrate
Copper sulphate
Magnesium sulphate
Manganese chloride
Sodium molydate
Zinc sulphate

Solution C

Sodium chloride

To 250 ml of solution add 900 ml of distilled water followed by 1.0 ml of solution B and 1.0 of solution C, and make up to 1 Litre (for 1 Kg of soil Preparation

2 COUNTING OF EOSINOPHILS

Equipment

Improved Neubaeur counting chambers
Eppendorf tubes
Eosinophil diluting fluid
100 microlitre pipette
10 microlitre pipette
Inverted microscope
Tally counter

Eosinophil diluting fluid

3ml formaldehyde buffer
8ml of 0.5% eosin Y solution (Sigma-AccustainTM)
Mix the above and make up to 100ml with distilled water
The solution is stored at 40 C

3 PBS-PI-METHIONINE

(200ml stock solution)

Methionine	20mg
PMSF	34.8mg in 1ml ethanol
TLCK	5mg
TPCK	10mg

4 COMPLETE CULTURE MEDIUM

RPMI 1640	194ml
Added by filtration:	
Penicillin	100units
Streptomycin	100µg

1mM L-Glutamine	2ml
Foetal calf serum (sterile, heat inactivated)	6ml
Amphotericin B (no filtration)	100µg

5 ELISA REAGENTS

Borate Buffered saline.

Boric acid	6.18 g
Di-sodium tetra borate	9.54 g
Sodium chloride	4.38 g
Distilled	to 1 litre pH. 8.2

PBS Tween 20

Tween 20 (Sigma, 70H0171)	0.5 g
PBS	1000 ml

Blocking solution (4% Normal Rabbit Serum/PBS Tween 20)

Rabbit Serum (Sigma, 115H8812)	10 ml
PBS Tween 20	240 ml

Washing solution (0.9% NaCl-Tween 20)

NaCl	9 g
Tween 20	0.5 g.
Distilled water	1 Litre

Phosphate Buffered Saline (PBS-A) pH 7.3

NaCl	10.11 g
KCl	0.362 g
KH_2PO_4	0.362 g
Na_2HPO_4	1Litre

Stopping solution (0.2 M H_2SO_4)

11 ml concentrated H_2SO_4 (Merck 1M) to 1000 ml distilled water (0.2 M)

6 SDS-PAGE SOLUTIONS

Solution A

1 M HCL	96 ml
Tris	73.2g
TEMED	92 µl
Deionized distilled H_2O to 200 ml	

Solution B

Acrylamide	150 g
Bis-acrylamide	4 g
Deionized distilled H_2O to 500 ml	

Stacking buffer

Tris	17.94 g
1 M HCL	144 ml
Deionized distilled H_2O to 10 ml	

Electrode running buffer

Tris base	15.0 g
Glycine	72.0 g
SDS	5.0 g

Deionized distilled water to 1 Litre.

Preparation of 10% separating gel

Deionized distilled H_2O	4.05
1.5 M Tris-HCL pH 8.8	2.50
10% w/v SDS	100 µl
30% w/v Acrylamide	3.30 ml
TEMED	5 µl
10% w/v Ammonium persulphate	50 µl

Preparation of 3% stacking gel

Deionized distilled water	6.10 ml
0.5 M Tris-HCL pH 6.8	2.50
10% w/v SDS	100 µl
30% w/v Acrylamide	10 µl
TEMED	10 µl
10% w/v Ammonium persulphate	100 µl

7 WESTERN BLOTTING

Western Blocking Buffer pH 7.4

Tris	12.1 g
NaCl	17.4 g
EDTA	0.74 g
Gelatin	5 g
NP 40	1 ml

Bjerrum and Schafer-Nielsen Transfer buffer

Tris	5.82 g
Glycine	2.93 g
SDS 10% w/v	3.75 ml
Methanol	200ml

Deionized distilled water to 1 L.

Reducing sample loading buffer

Deionized distilled water	4.0 ml
0.5 M Tris HCl pH 6.8	1.0 ml
Glycerol	0.8 ml
10 % w/v SDS	1.6 ml
ß- mercaptoethanol	0.4 ml
1% w/v bromophenol blue	0.2 ml

Appendix Table 4.1

Experiment 1: Sheep infected with F. hepatica (British and Peru strains)

Results of the Nematode faecal egg counts per gram of faeces (EPG), Fluke faecal egg counts per gram faeces (EPG) and Liveweight (Kg).

	Nematodes EPG						EPG Fluke						Liveweight (Kg)					
WPI	SH. 5	SH. 6	SH. 7	SH. 8	SH 9	SH.10	SH. 5	SH. 6	SH. 7	SH. 8	SH 9	SH.10	SH. 5	SH. 6	SH. 7	SH. 8	SH 9	SH.10
-2	200.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	19.0	23.0	22.0	25.5	78.0	53.0
-1	200.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	19.0	23.0	22.0	25.5	79.0	53.0
0	400.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	19.0	23.0	22.0	26.0	75.0	51.0
1	150.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	19.0	23.0	22.0	26.0	80.0	54.0
2	150.0	100.0	200.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0	23.0	22.0	26.0	79.0	56.0
3	300.0	100.0	200.0	200.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0	23.0	22.0	26.0	81.0	56.5
4	500.0	200.0	300.0	300.0	200.0	300.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0	23.0	23.0	26.0	81.0	57.0
5	450.0	150.0	330.0	300.0	100.0	300.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0	22.0	21.0	26.0	84.0	57.5
6	550.0	300.0	250.0	350.0	250.0	550.0	0.0	0.0	0.0	0.0	0.0	0.0	21.0	23.0	23.0	30.0	83.0	56.5
7	150.0	0.0	150.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	21.0	22.0	23.0	29.0	81.0	50.5
8	450.0	200.0	300.0	100.0	100.0	120.0	0.0	0.0	0.0	0.0	0.0	0.0	21.0	22.0	23.0	29.0	82.0	53.0
9	500.0	200.0	350.0	150.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	22.0	23.0	23.0	31.0	83.0	54.0
10	200.0	250.0	150.0	0.0	100.0	100.0	0.0	0.0	2.0	0.0	0.0	0.0	22.0	21.0	21.0	32.0	84.0	54.0
11	200.0	350.0	400.0	50.0	0.0	200.0	0.0	2.0	3.0	0.0	0.0	2.0	21.0	23.0	23.0	34.0	81.5	52.0
12		150.0	300.0	300.0	0.0	250.0		0.0	5.0	0.0	1.0	3.0		22.5	24.0	35.5	81.0	54.0
13		150.0	350.0	100.0	100.0	235.0		11.0	59.0	0.0	37.0	4.0		23.0	24.5	38.0	80.0	51.0
14		250.0	150.0	150.0	0.0	100.0		79.0	67.0	0.0	69.0	2.0		23.0	25.0	39.0	82.5	53.0
15		100.0	50.0	200.0	0.0	0.0		197.0	257.0	0.0	111.0	19.0		23.5	24.5	39.0	85.0	56.0
16		100.0	400.0	0.0	0.0	0.0		287.0	336.0	0.0	103.0	45.0		24.0	26.0	40.0	82.0	55.0
17		100.0	200.0	0.0	0.0	0.0		137.0	231.0	0.0	113.0	39.0		25.0	26.5	41.0	83.0	55.0

Appendix Table 4.2

Results of Packed cell volume (PCV) (%), Haemoglobulin (Hb) (g/l) levels and Red Blood Cell counts ($x10^{12}/l$)

	PCV						Hb						RBC					
WPI	SH. 5	SH. 6	SH. 7	SH. 8	Sh 9	sh.10	SH. 5	SH. 6	SH. 7	SH. 8	SH 9	SH.10	SH. 5	SH. 6	SH. 7	SH. 8	SH 9	SH.10
-2	21	32	30	29	32	32	8.4	13.1	13.2	12.0	12.1	14.0	9.3	13.7	12.5	11.3	11.8	11.7
-1	22	31	28	30	34	31	9.1	13.8	12.5	12.2	11.8	13.5	8.7	11.4	12.2	12.8	10.8	12.2
0	22	31	28	30	33	32	8.9	13.9	12.0	12.2	12.5	12.9	8.7	11.5	12.2	13.0	10.8	13.2
1	20	29	33	31	32	32	8.6	12.3	13.9	13.4	11.7	13.4	9.2	10.8	12.8	13.6	10.9	13.7
2	21	33	31	31	32	31	10.0	13.1	12.0	13.6	14.3	13.8	9.5	12.1	9.6	13.7	10.4	11.4
3	21	27	30	29	33	29	8.9	12.9	11.9	13.1	13.5	13.2	9.6	10.7	11.5	12.4	10.6	11.5
4	21	28	30	29	29	31	9.8	11.0	11.2	13.4	13.1	12.2	11.3	10.0	12.5	12.4	11.3	10.8
5	21	26	29	29	30	33	9.7	11.0	11.6	12.4	13.1	13.3	11.7	12.7	13.4	12.2	12.6	12.1
6	25	24	27	31	29	29	9.8	9.6	10.7	12.9	13.0	13.2	13.1	12.7	12.0	12.4	12.6	12.0
7	21	23	28	29	28	29	9.0	9.2	10.6	12.9	12.7	13.0	9.5	9.2	10.7	12.1	9.794	10.7
8	20	20	23	32	27	32	8.5	8.1	9.3	13.1	12.1	12.1	10.8	8.4	8.3	12.5	9.141	8.3
9	19	17	22	28	28	31	7.9	6.8	8.9	12.3	12.9	15.0	7.4	6.3	8.4	12.3	7.360	8.4
10	16	15	22	28	26	32	5.9	6.2	8.8	11.7	14.6	13.3	8.2	5.4	8.3	12.7	7.313	8.3
11	15	15	19	28	28	31	5.1	5.8	7.0	11.6	13.1	14.1	4.9	5.4	6.6	11.0	5.640	9.0
12		14	21	29	27	30		5.5	8.8	12.8	13.6	10.8		5.6	7.7	11.6	6.649	7.8
13		14	23	29	28	33		6.6	8.8	13.9	13.1	13.2		4.4	7.7	10.2	6.043	7.4
14		14	24	30	27	31		5.9	11.2	13.9	14.0	14.8		4.1	5.9	13.1	4.996	8.0
15		14	23	29	27	35		6.8	10.6	14.0	12.7	14.5		4.5	8.4	11.0	6.481	8.3
16		14	21	30	27	30		6.6	8.7	13.8	11.9	12.7		3.8	8.4	11.2	6.130	8.4
17		14	22	29	27	32		5.7	9.2	12.8	12.6	13.1		6.7	8.7	11.2	7.727	8.3

Appendix Table 4.3

Results of MCV (fl), MCH (pg) and MCHC (g/dl)

	MCV						MCH						MCHC					
WPI	SH. 5	SH. 6	SH. 7	SH. 8	Sh 9	sh.10	SH. 5	SH. 6	SH. 7	SH. 8	Sh 9	sh.10	SH. 5	SH. 6	SH. 7	SH. 8	Sh 9	sh.10
-2	23.0	23.0	23.0	26.0	27.1	27.4	9.0	10.0	11.0	11.0	10.20	12.00	40	31	44	41	38	44
-1	25.0	27.0	23.0	23.0	31.5	25.5	10.0	12.0	10.0	10.0	10.90	11.10	41	45	45	41	35	44
0	25.0	27.0	23.0	23.0	30.6	24.2	10.0	12.0	10.0	9.0	11.60	9.80	40	45	43	41	38	40
1	22.0	27.0	26.0	23.0	29.2	23.4	9.0	11.0	11.0	10.0	10.70	9.80	43	42	42	43	37	42
2	22.0	27.0	32.0	23.0	30.7	27.2	10.5	11.0	12.0	10.0	13.70	12.10	48	40	39	44	45	45
3	22.0	25.0	26.0	23.0	31.1	25.3	9.0	12.0	10.0	10.5	12.70	11.50	42	48	40	45	41	46
4	19.0	28.0	24.0	23.0	25.7	28.8	9.0	11.0	9.0	10.0	11.60	11.30	47	39	37	43	45	39
5	18.0	15.0	22.0	24.0	23.8	27.2	8.0	6.0	9.0	10.0	10.40	11.00	46	42	40	43	44	40
6	19.0	19.0	22.0	25.0	23.0	24.1	8.0	8.0	9.0	10.0	10.30	11.00	39	40	40	42	45	46
7	22.0	25.0	17.0	20.6	28.6	27.2	9.5	10.0	6.0	9.0	13.00	12.20	43	40	38	44	45	45
8	16.0	24.0	28.0	25.5	29.5	38.5	7.0	10.0	11.0	10.5	13.20	14.60	43	41	10	41	45	38
9	26.0	27.0	26.0	23.0	38.0	36.9	11.0	11.0	11.0	10.0	17.50	17.80	42	40	40	44	46	48
10	19.0	28.0	26.0	22.0	35.6	38.4	7.0	11.5	10.6	9.0	20.00	16.00	37	41	40	42	56	42
11	30.0	27.0	29.0	25.0	49.6	34.4	10.0	11.0	10.7	10.5	23.20	15.70	34	39	37	41	47	45
12		25.0	27.0	25.0	40.6	38.6		10.0	11.5	11.0	20.50	13.90		39	42	44	50	36
13		32.0	20.0	28.0	46.3	44.5		15.0	8.0	14.0	21.70	17.80		47	38	48	47	40
14		34.0	27.0	20.0	54.0	38.7		14.5	12.5	9.0	28.00	18.50		42	47	46	52	48
15		31.0	27.0	26.0	41.7	42.1		15.0	12.5	13.0	19.60	17.50		49	46	48	47	41
16		36.5	32.7	27.0	44.0	35.7		17.0	14.0	12.0	19.40	15.10		47	41	46	44	42
17		29.5	39.0	26.0	34.9	38.4		12.0	16.0	11.5	16.30	15.70		41	42	44	47	41

Appendix Table 4.4

Results of total White Blood Cells and Eosinophils Counts ($x10^{12}/l$).

	White Blood Cells (WBC)						Eosinophils Counts					
WPI	SH. 5	SH. 6	SH. 7	SH. 8	SH 9	SH.10	SH. 5	SH. 6	SH. 7	SH. 8	SH 9	SH.10
-2	4.412	8.621	7.121	4.781	5.400	4.850	0.013	0.294	0.094	0.063	0.000	0.013
-1	5.012	7.600	6.800	5.600	5.400	5.100	0.006	0.063	0.031	0.044	0.033	0.006
0	4.300	7.600	6.600	5.200	5.352	5.312	0.025	0.069	0.038	0.050	0.043	0.038
1	5.200	8.500	6.000	5.700	5.500	6.500	0.038	0.075	0.044	0.050	0.344	1.238
2	9.700	9.800	6.200	5.100	7.700	7.600	0.684	0.825	0.569	0.044	1.875	1.425
3	10.000	10.400	8.750	5.400	10.310	7.440	1.962	2.175	2.856	0.019	4.169	1.913
4	9.900	9.300	10.100	5.400	9.950	10.640	3.125	1.863	3.281	0.031	1.388	3.419
5	10.500	8.300	9.960	5.300	13.040	11.650	2.725	1.688	0.875	0.013	2.313	3.238
6	9.960	9.670	11.980	5.720	13.761	9.627	2.563	1.938	2.156	0.006	7.062	1.344
7	8.490	9.190	14.080	6.300	15.633	9.957	1.450	2.756	3.025	0.019	7.181	2.494
8	8.750	10.730	13.870	5.880	13.745	8.432	2.069	1.350	2.125	0.043	5.388	2.137
9	9.710	8.920	13.870	5.900	15.438	10.825	2.963	2.844	3.669	0.013	7.738	2.069
10	9.293	9.979	15.507	6.098	15.721	8.980	2.362	3.250	5.419	0.006	8.306	2.65
11	14.000	8.987	15.288	5.744	14.022	9.080	0.862	2.644	5.711	0.006	5.785	2.325
12		9.484	18.166	4.810	13.211	9.747		2.906	5.575	0.006	6.262	2.281
13		10.010	15.080	6.360	14.126	10.678		2.194	4.375	0.019	6.631	2.619
14		8.718	12.895	6.079	11.278	8.280		2.681	5.881	0.044	1.880	1.256
15		11.423	14.134	5.557	9.442	7.990		4.488	5.213	0.013	1.388	1.188
16		9.415	10.633	6.027	10.017	7.883		3.218	2.706	0.013	2.350	0.925
17		8.564	9.491	6.251	9.526	7.697		2.025	1.531	0.050	0.712	0.587

Appendix Table 4.5

Results of Diferential Neutrophils, Lymphocytes counts ($x10^9/l$) and Monocytes.

	Neutrophil.						Lymphocytes.						Monocytes.					
WPI	SH.5	SH. 6	SH.7	SH.8	SH.9	SH.10	SH.5	SH. 6	SH.7	SH.8	SH.9	SH.10	SH.5	SH. 6	SH.7	SH.8	SH.9	SH.10
-2	2.200	0.602	3.053	1.728	2.268	1.504	2.156	7.310	3.763	2.928	2.700	2.862	0.044	0.172	0.142	0.096	0.108	0.194
-1	3.250	0.836	1.496	2.520	1.944	2.142	1.650	6.080	4.828	2.520	3.132	2.601	0.100	0.608	0.340	0.112	0.108	0.102
0	3.016	4.420	2.880	3.534	3.158	1.912	1.872	3.570	2.760	1.881	1.766	2.922	0.208	0.510	0.300	0.171	0.107	0.053
1	1.978	0.456	1.452	1.768	2.365	1.885	2.193	6.156	4.620	2.964	2.145	3.575	0.129	0.988	0.462	0.416	0.110	0.260
2	3.492	0.392	0.868	1.581	3.696	1.596	4.074	8.330	4.402	3.111	2.772	3.724	0.388	0.392	0.124	0.357	0.308	0.228
3	5.500	0.416	[illegible]3	2.322	4.227	1.488	2.000	6.136	4.725	2.808	4.330	4.166	0.900	0.312	0.438	0.270	0.206	0.521
4	3.332	1.395	1.111	1.944	2.687	2.341	2.744	5.301	4.646	3.078	2.587	5.214	0.686	0.558	0.505	0.378	0.697	0.000
5	4.410	1.162	1.693	1.325	2.869	2.330	2.730	5.146	5.478	3.604	4.173	5.592	0.630	0.083	0.797	0.371	0.782	0.233
6	2.689	1.257	1.318	2.288	1.927	2.599	2.789	5.995	7.547	3.146	4.541	4.140	0.797	0.097	0.240	0.172	0.688	0.096
7	3.396	0.735	2.675	1.764	2.189	2.290	3.566	4.779	6.054	3.906	6.722	4.680	0.425	0.368	0.141	0.567	0.313	0.100
8	1.838	1.180	1.537	1.940	2.062	1.939	2.800	5.150	6.426	3.352	5.086	4.806	0.263	0.751	0.699	0.294	0.275	0.169
9	3.395	1.068	1.664	1.534	2.933	2.815	1.940	3.115	5.825	3.953	6.484	5.521	0.679	0.623	0.000	0.295	0.618	0.000
10	4.368	1.098	2.636	1.098	2.358	2.604	1.859	5.588	6.668	4.574	5.974	3.592	0.651	0.200	0.775	0.244	0.314	0.449
11	7.140	1.528	2.752	1.379	1.542	2.542	5.180	3.954	3.516	4.193	5.609	3.814	0.840	0.270	0.764	0.172	0.000	0.091
12		1.233	6.176	1.580	1.453	2.729		4.837	5.631	5.170	5.284	5.068		0.000	0.000	0.287	0.132	0.292
13		2.202	4.222	3.053	2.401	2.136		3.203	5.127	3.116	6.357	6.941		0.200	0.452	0.191	0.141	0.214
14		1.221	1.418	1.898	3.947	2.898		4.795	5.287	4.745	6.090	4.968		0.262	0.129	0.068	0.226	0.994
15		1.143	1.696	1.334	1.133	1.199		4.230	7.067	3.945	6.421	5.993		0.114	0.424	0.167	0.189	0.799
16		2.354	3.403	1.025	2.905	1.577		4.331	5.104	3.918	5.309	5.124		0.094	0.319	1.085	0.100	0.473
17		0.856	1.519	1.750	1.143	1.155		5.395	5.220	4.251	6.478	5.773		0.257	0.380	0.250	0.191	0.77

Appendix Table 4.6

Results of Diferential Eosinophil and Basophil counts in ($x10^9/l$)

	Eosinophil.						Basophil.					
WPI	SH.5	SH. 6	SH.7	SH.8	SH.9	SH.10	SH.5	SH. 6	SH.7	SH.8	SH.9	SH.10
-2	0.000	0.516	0.071	0.048	0.324	0.243	0.000	0.000	0.071	0.000	0.000	0.049
-1	0.000	0.076	0.068	0.056	0.216	0.204	0.000	0.000	0.068	0.056	0.000	0.051
0	0.052	0.000	0.060	0.057	0.321	0.372	0.052	0.000	0.000	0.057	0.000	0.053
1	0.000	0.000	0.000	0.052	0.495	1.105	0.000	0.000	0.066	0.000	0.055	0.065
2	1.746	0.980	0.806	0.051	0.847	2.052	0.000	0.000	0.062	0.000	0.077	0.000
3	1.600	3.536	2.975	0.000	1.547	1.265	0.000	0.000	0.000	0.000	0.000	0.000
4	3.038	2.046	3.737	0.000	3.980	2.979	0.000	0.000	0.000	0.000	0.100	0.000
5	2.730	1.909	1.992	0.000	5.086	3.379	0.000	0.000	0.000	0.000	0.130	0.117
6	3.685	2.321	2.875	0.114	6.605	2.696	0.000	0.000	0.000	0.000	0.000	0.096
7	1.104	3.308	5.210	0.063	6.253	2.888	0.000	0.000	0.000	0.000	0.156	0.000
8	3.763	3.541	5.169	0.294	6.185	1.433	0.088	0.107	0.140	0.000	0.137	0.084
9	3.589	3.827	6.242	0.059	5.403	2.490	0.097	0.267	0.139	0.059	0.000	0.000
10	2.416	3.093	5.427	0.183	7.074	2.335	0.000	0.000	0.000	0.000	0.000	0.000
11	1.120	3.235	8.256	0.000	6.871	2.633	0.000	0.000	0.000	0.000	0.000	0.000
12		3.414	6.358	0.144	6.341	1.657		0.000	0.000	0.000	0.000	0.000
13		4.404	4.976	0.000	5.227	1.388		0.000	0.000	0.000	0.000	0.000
13		2.441	6.061	0.068	0.113	0.083		0.000	0.000	0.000	0.000	0.000
14		5.945	4.947	0.111	1.700	0.000		0.000	0.000	0.000	0.000	0.000
15		2.636	1.808	0.000	1.703	0.709		0.000	0.000	0.000	0.000	0.000
16		2.055	2.373	0.000	1.715	0		0.000	0.000	0.000	0.000	0.000

Appendix Table 4.7

Results of serum Albumin (g/l), Total Protein (g/dl) and Glucose (mmol/l)

	Albumin						Total Protein						Glucose					
WPI	Sh.5	Sh. 6	Sh. 7	Sh. 8	Sh. 9	Sh. 10	Sh.5	Sh. 6	Sh. 7	Sh. 8	Sh. 9	Sh. 10	Sh.5	Sh. 6	Sh. 7	Sh. 8	Sh. 9	Sh.10
-2	31.00	37.00	40.00	38.00	36.1	48.2	5.30	7.30	6.20	8.10	7.6	7.2	2.75	2.5	3.02	2.41	2.24	2.4
-1	24.00	40.00	32.00	37.00	37.1	35.8	6.40	7.30	6.30	6.60	8.0	8.0	2.12	2.1	2.58	2.22	2.35	2.7
0	30.00	36.00	30.00	36.00	37.6	34.9	6.60	6.20	7.90	8.40	8.0	7.2	1.64	2.2	2.46	3	2.61	2.4
1	27.00	36.00	31.00	36.00	34.0	38.6	6.90	6.00	7.20	6.60	7.4	7.5	2.61	2.6	2.4	3.12	2.61	2.5
2	33.00	38.00	35.00	38.00	40.9	35.7	7.00	6.50	6.90	7.00	7.9	7.1	2.3	3	2.5	2.59	2.18	2
3	30.00	30.00	41.00	34.00	34.0	29.9	7.70	8.80	10.00	8.00	7.3	7.0	1.95	3	2.22	2.47	2.25	2.6
4	33.00	32.30	30.00	38.00	38.5	35.8	7.40	6.80	8.40	8.60	8.0	7.5	1.75	2.8	1.85	2.5	2.06	1.7
5	30.00	31.50	32.00	35.00	31.1	38.0	7.70	8.60	8.80	8.00	7.4	7.6	2.18	1.27	2.43	2.53	2.5	2.2
6	40.00	38.00	32.00	37.00	39.5	37.0	8.10	8.00	8.20	7.40	7.6	7.9	1.84	1.44	2.31	2.8	2.1	2.61
7	39.00	38.00	43.00	34.00	40.4	38.3	6.60	7.40	7.40	8.90	8.3	7.7	2.25	1.13	1.66	2.61	2.2	2.18
8	43.00	35.00	33.60	40.00	35.7	33.4	8.40	8.90	7.30	8.40	7.8	7.7	1.85	1.03	1.74	2.75	2.5	2.25
9	35.00	36.00	32.00	39.00	39.8	37.2	7.70	8.40	8.60	8.20	7.9	8.3	2.06	1.57	1.42	2.61	3	2.06
10	23.00	33.00	34.00	40.00	44.1	32.9	7.70	8.20	8.90	7.4	7.4	7.7	0.83	1.27	1.3	2.43	2.9	2.5
11	19.00	33.00	25.00	36.00	38.9	33.9	4.40	12.90	7.20	7.6	8.7	8.3	1.2	1.44	1.43	2.31	2.8	2.1
12	24.00	31.00	25.00	36.00	31.3	30.3	5.10	9.20	8.20	8.3	8.9	8.0	1.06	1.13	1.82	2.61	2.42	2.2
13		21.00	24.00	38.00	34.0	31.1		8.90	6.30	7.8	8.6	7.8		1.03	1.67	2.61	2.43	2.6
14		22.20	26.00	36.00	32.1	30.6		6.10	8.00	7.9	8.9	8.3		1.42	2.32	3	2.31	3
15		21.00	21.00	33.00	29.5	29.9		6.80	7.00	8.9	8.3	7.9		1.3	2.1	3.1	2.43	3
16		17.00	21.00	30.00	28.5	31.3		6.00	6.00	8.6	7.6	7.8		1.43	1.6	2.8	2.31	2.8
17		17.00	22.00	34.00	30.4	30.6		6.00	5.20	8.9	7.6	7.3		1.6	1.89	2.9	2.32	2.42

Appendix Table 4.8

Results of Gamma Glutamyltransferase (IU), Glutamate Dehydrogenase (IU) and b-Hydroxyl-butyrate (mmol)

	γGT						GLDH						b-Hydroxyl-butyrate					
WPI	Sh.5	Sh. 6	Sh. 7	Sh. 8	Sh. 9	Sh. 10	Sh.5	Sh. 6	Sh. 7	Sh. 8	Sh. 9	Sh. 10	Sh.5	Sh. 6	Sh. 7	Sh. 8	Sh. 9	Sh. 10
-2	27.40	20.10	20.80	21.20	24.7	26.6	0.20	0.20	0.20	3.90	1.4	8.7	0.31	0.29	0.35	0.35	0.35	0.29
-1	35.10	27.00	20.80	27.00	30.9	24.7	0.20	0.60	0.20	1.80	2.2	3	0.43	0.43	0.4	0.31	0.29	0.32
0	41.00	21.20	18.50	24.00	37.4	29.7	0.60	2.60	0.60	0.90	0.4	5.9	0.48	0.46	0.38	0.32	0.36	0.36
1	35.50	26.20	29.70	25.50	28.6	27	0.60	0.90	0.40	0.80	1.2	3.3	0.49	0.45	0.44	0.34	0.32	0.32
2	40.90	29.30	32.40	26.60	26.2	25.1	3.70	4.90	31.30	2.60	9.3	4.10	0.45	0.46	0.42	0.3	0.41	0.39
3	38.60	39.00	39.50	28.60	34.7	24.5	32.50	141.60	141.20	1.80	120	126.10	0.58	0.52	0.41	0.29	0.29	0.38
4	37.40	46.70	39.00	22.00	28.2	25.5	104.00	149.10	146.60	2.00	91.40	54.40	0.47	0.51	0.35	0.29	0.32	0.35
5	46.70	57.50	39.00	23.20	30.9	25.9	99.90	143.80	151.90	2.00	109	78.40	0.51	0.55	0.31	0.32	0.34	0.34
6	57.00	63.70	41.70	25.50	35.1	33.9	110.70	138.50	141.60	3.30	113.2	91.40	0.42	0.21	0.31	0.36	0.22	0.31
7	40.50	56.30	42.80	23.50	30.5	40	52.00	126.10	147.60	6.10	98.8	88.30	0.39	0.27	0.25	0.32	0.25	0.37
8	44.40	55.20	40.50	24.70	36	38	54.40	88.30	144.60	3.70	97.9	65.60	0.49	0.33	0.36	0.29	0.29	0.31
9	55.60	50.00	49.40	28.10	38.7	43	78.40	65.60	150.50	4.50	88.2	80.00	0.42	0.43	0.41	0.29	0.24	0.3
10	63.70	51.70	66.40	32.00	40	59	91.40	80.00	131.20	12.80	69.5	52.00	0.25	0.5	0.4	0.31	0.27	0.32
11	77.20	75.70	65.20	34.70	44	56.7	79.20	74.90	105.00	6.10	89.3	32.9	0.36	0.58	0.41	0.31	0.25	0.36
12	79.90	93.00	52.50	30.00	98.9	51.1	99.50	31.70	47.90	9.50	86.2	18.7	0.33	0.32	0.43	0.25	0.2	0.31
13		77.00	54.00	25.90	67.5	78.1		54.60	63.20	14.00	29.7	34.3		0.58	0.47	0.29	0.18	0.25
14		72.90	50.20	27.00	79.5	98.4		32.90	50.40	4.10	80.4	54.2		0.87	0.58	0.31	0.21	0.3
15		62.90	44.80	28.00	64.5	78.4		42.60	70.70	7.10	31.5	36.1		0.53	0.45	0.31	0.26	0.28
16		46.30	36.30	26.60	93.8	45.9		43.70	36.80	7.50	42.4	36.8		0.68	0.48	0.25	0.24	0.32
17		33.60	37.80	28.50	61.8	38.2		49.30	37.00	0.60	10	37.8		0.7	0.55	0.3	0.3	0.31

Appendix Table 9:

Glucose and β-Hydroxyl-Butyrate (mmol/ml) values in sheep 24, 26, 28 and 30 infected with *F. hepatica* (Britsh strain) and sheep 22 and 32 as uninfected controls.

	Glucose (mmol/ml)						β-Hydroxyl-Butyrate (mmol/ml)					
WPI	SH. 24	SH. 26	SH. 28	SH. 30	SH. 22	SH. 32	SH.24	SH. 26	SH. 28	SH. 30	SH. 22	SH. 32
-2	3.33	3.50	3.60	3.20	2.20	2.24	0.47	0.43	0.37	0.48	0.35	0.27
-1	2.78	2.90	3.10	2.26	2.08	3.00	0.36	0.66	0.47	0.31	0.32	0.29
0	2.12	1.80	2.50	1.93	3.03	2.50	0.37	0.37	0.27	0.42	0.40	0.35
1	1.55	1.90	2.30	1.79	2.20	2.60	0.57	0.43	0.35	0.30	0.31	0.35
2	2.65	1.90	2.80	1.31	1.40	2.08	0.41	0.41	0.42	0.39	0.46	0.39
3	2.02	1.60	2.40	2.07	2.30	3.03	0.37	0.40	0.54	0.39	0.49	0.38
4	1.60	2.20	2.10	1.79	3.00	1.17	0.27	0.44	0.41	0.35	0.38	0.40
5	1.66	2.00	1.60	1.99	2.50	3.04	0.51	0.53	0.38	0.33	0.40	0.45
6	2.31	2.00	1.80	1.78	2.60	2.31	0.62	0.57	0.26	0.41	0.45	0.36
7	3.03	2.10	1.60	1.55	2.90	2.57	0.41	0.50	0.39	0.38	0.36	0.48
8	2.04	2.40	2.20	1.81	2.70	2.59	0.45	0.52	0.69	0.70	0.59	0.39
9	2.81	1.80	2.60	2.10	2.90	2.43	0.76	0.61	0.49	0.63	0.58	0.54
10	1.46	2.20	1.80	1.66	3.50	2.25	0.48	0.48	0.88	0.53	0.38	0.35
11	2.24	2.10	2.10	2.14	2.70	2.05	0.49	0.54	0.50	0.29	0.40	0.34
12	2.41	1.50	1.90	1.77	2.40	2.06	0.76	0.52	0.50	0.47	0.45	0.38
13	1.65	1.60	1.70	1.19	2.40	2.38	0.35	0.41	0.39	0.50	0.36	0.40
14	1.57	1.90	2.30	1.57	2.80	2.40	0.41	0.49	0.56	0.41	0.38	0.45
15	2.30	1.90	1.90	1.98	3.60	2.80	0.76	0.50	0.45	0.57	0.36	0.36
16	1.94	1.20	1.10	1.61	3.10	3.60	0.41	0.42	0.52	0.29	0.31	0.45
17	0.93	2.20	2.00	1.33	2.40	2.01	0.31	0.36	0.41	0.41	0.31	0.48
18	1.97	1.80	2.10	1.69	2.10	3.60	0.37	0.45	0.62	0.31	0.37	0.50
19	1.53	1.60	1.30	1.66	2.60	3.10	0.31	0.52	0.41	0.56	0.38	0.27
20	1.47	2.20	2.10	1.51	3.10	2.50	0.32	0.26	0.36	0.41	0.40	0.27
21	1.82	2.30	2.00	1.85	4.30	2.30	0.32	0.25	0.42	0.28	0.45	0.47
22	1.50	1.80	1.90	1.60	2.80	2.80	0.28	0.30	0.38	0.38	0.36	0.38

Experiment 3: Five sheep infected with 100 Cysts of *F. gigantica* and four uninfected controls Sheep infected with

Appendix Table 4.10

Egg counts per gram (EPG) in five sheep infected with 100 Cysts of F. gigantica and four uninfected controls

		Infefected group						Uninfected control				
Date	WPI	SH. 11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)
31.03.94	-2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
07.04.94	-1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14.04.94	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
21.04.94	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
28.04.94	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
05.05.94	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12.05.94	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
19.05.94	5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
26.05.94	6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
02.06.94.	7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
09.06.94	8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16.06.94	9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
23.06.94	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
30.06.94	11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
07.07.94	12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14.07.94	13	0.0	0.0	0.0	0.0	1.0	0.2	0.0	0.0	0.0	0.0	0.0
21.07.94	14	0.0	0.0	0.0	1.0	21.0	4.4	0.0	0.0	0.0	0.0	0.0
28.07.94	15	0.0	1.0	0.0	8.0	67.0	15.2	0.0	0.0	0.0	0.0	0.0
04.08.94	16	0.0	5.0		43.0	96.0	36.0	0.0	0.0	0.0	0.0	0.0
11.08.94	17	0.0	23.0		70.0	92.0	46.3	0.0	0.0	0.0	0.0	0.0
18.08.94	18	2.0	22.0		92.0	141.0	64.3	0.0	0.0	0.0	0.0	0.0
25.08.94	19	3.0	54.0		50.0	32.0	34.8	0.0	0.0	0.0	0.0	0.0
01.09.94	20	2.0	100.0		173.0	160.0	108.8	0.0	0.0	0.0	0.0	0.0
08.09.94	21	16.0	195.0		181.0	188.0	145.0	0.0	0.0	0.0	0.0	0.0
15.09.94	22	21.0	74.0		81.0	16.0	48.0	0.0	0.0	0.0	0.0	0.0
22.09.94	23	67.0	192.0		280.0	206.0	186.3	0.0	0.0	0.0	0.0	0.0
29.09.94	24	90.0	175.0		236.0	4.1	126.3	0.0	0.0	0.0	0.0	0.0
06.10.94.	25	85.0	174.0		211.0	61.0	132.8	0.0	0.0	0.0	0.0	0.0
13.10.94	26							0.0	0.0	0.0	0.0	0.0

Appendix Table 4.11

Mean Liveweight of five sheep infected with 100 Cysts of F. gigantica and four uninfected controls

	Infefected group							Uninfected control				
Date	WPI	SH. 11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)
31.03.94	-2	51.0	39.0	43.0	41.0	42.0	43.2	40.0	41.0	43.0	47.0	42.8
14.04.94	0	51.0	40.0	44.0	41.5	42.0	43.7	40.0	41.0	43.0	47.0	42.8
28.04.94	2	50.0	40.0	44.0	42.0	44.5	44.1	41.0	42.0	*44.0*	49.0	44.0
12.05.94	4	51.0	40.0	45.0	41.0	45.0	44.4	42.0	43.0	45.0	49.5	44.9
26.05.94	6	51.5	41.0	45.0	42.0	45.0	44.9	43.0	45.5	45.0	50.0	45.9
09.06.94	8	52.0	42.0	45.0	43.0	45.0	45.4	43.0	46.0	46.5	51.0	46.6
23.06.94	10	51.0	42.0	44.0	45.0	46.0	45.6	43.5	46.0	46.0	52.0	46.9
07.07.94	12	51.0	43.0	48.0	47.0	47.0	47.2	44.0	47.0	46.0	53.0	47.5
21.07.94	14	51.0	43.0	49.0	48.0	48.0	47.8	44.0	47.0	47.0	53.5	47.9
04.08.94	16	52.0	43.5		45.5	45.0	46.5	45.0	48.0	47.5	54.0	48.6
18.08.94	18	53.0	44.0		46.0	46.0	47.3	46.0	49.0	48.0	55.0	49.5
01.09.94	20	51.0	43.0		47.0	45.5	46.6	47.0	*50.0*	49.0	56.0	50.5
15.09.94	22	51.0	45.0		48.0	47.0	47.8	47.0	*51.0*	48.5	57.0	50.9
29.09.94	24	53.0	45.0		49.5	48.0	48.9	46.5	*51.5*	50.0	58.0	51.5

Appendix 4.12

Total Red blood Cells levels ($x10^{12}/l$) in

		Infected group								Uninfected group						
Date	WPI	SH.11	SH.12	SH.13	SH.14	SH.15	Mean (+)	StDev	SEM	SH.16	SH.17	SH.18	SH.19	Mean (-)	StDev	SEM
31.03.94	-2	14.080	11.440	13.430	11.410	10.070	12.086	1.637	0.732	10.770	9.610	9.150	11.130	10.165	0.937	0.469
07.04.94	-1	11.330	11.540	15.500	12.130	11.910	12.482	1.716	0.767	13.640	12.140	11.860	11.540	12.295	0.930	0.465
14.04.94	0	10.240	9.770	13.450	13.320	10.460	11.448	1.786	0.799	14.100	12.950	10.140	12.790	12.495	1.675	0.837
21.04.94	1	10.370	12.170	14.000	13.200	12.660	12.480	1.361	0.609	11.330	12.480	9.420	12.470	11.425	1.442	0.721
28.04.94	2	14.630	15.750	12.870	13.510	12.470	13.846	1.342	0.600	11.500	12.650	14.050	12.500	12.675	1.049	0.525
05.05.94	3	13.100	14.740	13.150	16.160	12.380	13.906	1.527	0.683	12.800	13.580	12.580	13.230	13.047	0.446	0.223
12.05.94	4	12.540	11.190	12.570	14.430	11.790	12.504	1.220	0.546	13.420	12.850	11.610	12.050	12.483	0.809	0.404
19.05.94	5	11.660	11.490	12.410	12.430	11.180	11.834	0.562	0.251	12.560	12.150	10.200	11.440	11.587	1.034	0.517
26.05.94	6	11.720	11.530	12.270	12.430	11.170	11.824	0.522	0.234	12.810	11.780	11.750	12.410	12.187	0.515	0.257
02.06.94.	7	12.590	11.850	12.220	11.670	9.150	11.496	1.358	0.607	12.480	12.680	10.070	10.410	11.410	1.361	0.680
09.06.94	8	12.930	9.540	12.620	13.230	9.770	11.618	1.807	0.808	13.470	14.120	10.810	11.640	12.510	1.545	0.772
16.06.94	9	12.080	8.850	10.890	13.580	10.440	11.168	1.777	0.795	12.300	10.580	11.370	10.750	11.250	0.778	0.389
23.06.94	10	10.320	8.890	11.070	12.370	9.410	10.412	1.378	0.616	10.780	10.780	9.460	11.110	10.532	0.732	0.366
30.06.94	11	10.320	8.740	10.610	12.170	8.850	10.138	1.414	0.632	11.480	11.000	9.720	10.440	10.660	0.757	0.379
07.07.94	12	8.920	8.990	10.580	12.050	7.570	9.622	1.726	0.772	11.320	10.840	10.340	10.140	10.660	0.529	0.265
14.07.94	13	9.810	8.150	8.960	11.900	6.970	9.158	1.856	0.830	10.410	10.120	9.650	10.310	10.122	0.337	0.169
21.07.94	14	8.880	8.430	9.000	10.430	7.330	8.814	1.118	0.500	10.570	10.010	10.210	10.490	10.320	0.258	0.129
28.07.94	15	9.750	6.980	7.670	8.440	8.050	8.178	1.031	0.461	11.650	10.650	10.400	10.420	10.780	0.591	0.295
04.08.94	16	9.810	6.870		6.830	7.580	7.773	1.401	0.701	11.190	10.320	10.010	10.170	10.422	0.527	0.264
11.08.94	17	9.430	7.170		7.500	6.880	7.745	1.152	0.576	10.850	10.370	10.150	11.390	10.690	0.551	0.275
18.08.94	18	8.940	10.000		9.700	8.430	9.267	0.715	0.357	13.890	12.400	12.470	12.620	12.845	0.703	0.351
25.08.94	19	8.660	8.000		9.430	7.500	8.398	0.836	0.418	12.070	10.710	11.220	11.430	11.358	0.563	0.282
01.09.94	20	3.060	7.610		9.120	8.680	7.120	2.780	1.390	11.860	10.220	10.250	11.290	10.905	0.808	0.404
08.09.94	21	4.120	7.850		9.680	7.230	7.220	2.310	1.160	10.650	9.660	10.020	10.850	10.295	0.552	0.276
15.09.94	22	7.520	7.750		9.290	7.270	7.957	0.910	0.455	11.140	9.080	9.270	11.170	10.165	1.146	0.573
22.09.94	23	9.420	7.640		9.560	6.510	8.283	1.470	0.735	11.210	8.970	9.860	10.500	10.135	0.952	0.476
29.09.94	24	9.300	6.620		9.520	7.260	8.175	1.453	0.726	11.260	9.470	10.160	10.710	10.400	0.766	0.383
06.10.94.	25	9.030	7.650		9.860	6.290	8.207	1.570	0.785	11.650	9.830	10.550	10.850	10.720	0.753	0.377
13.10.94	26				11.110	6.090	8.600	3.550	2.510			10.320	10.470	10.395	0.106	0.075

Appendix Table 4.13

Packed Cell Volume (PCV) (%)

		Infected group								Uninfected group						
Date	WPI	SH. 11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	StDev	SEM	SH. 16	SH. 17	SH. 18	SH.19	Mean (-)	StDev	SEM
31.03.94	-2	34.0	33.0	40.0	34.0	28.0	33.8	4.3	1.9	35.0	31.0	29.0	36.0	32.8	3.3	1.7
07.04.94	-1	38.0	37.0	39.0	36.0	29.0	35.8	4.0	1.8	37.0	29.0	29.0	34.0	32.3	4.0	2.0
14.04.94	0	39.0	32.0	38.0	35.0	25.0	33.8	5.6	2.5	38.0	36.0	27.0	37.0	34.5	5.1	2.5
21.04.94	1	38.0	37.0	36.0	34.0	28.0	34.6	4.0	1.8	38.0	37.0	31.0	37.0	35.8	3.2	1.6
28.04.94	2	34.0	38.0	36.0	38.0	28.0	34.8	4.2	1.9	38.0	38.0	31.0	37.0	36.0	3.4	1.7
05.05.94	3	38.0	37.0	38.0	39.0	26.0	35.6	5.4	2.4	39.0	38.0	30.0	36.0	35.8	4.0	2.0
12.05.94	4	36.0	34.0	34.0	38.0	26.0	33.6	4.6	2.0	38.0	37.0	28.0	36.0	34.8	4.6	2.3
19.05.94	5	35.0	39.0	33.0	36.0	29.0	34.4	3.7	1.7	40.0	39.0	28.0	38.0	36.3	5.6	2.8
26.05.94	6	38.0	36.0	34.0	35.0	30.0	34.6	3.0	1.3	39.0	38.0	32.0	38.0	36.8	3.2	1.6
02.06.94.	7	36.0	38.0	35.0	36.0	29.0	34.8	3.4	1.5	39.0	36.0	32.0	39.0	36.5	3.3	1.7
09.06.94	8	37.0	35.0	35.0	36.0	29.0	34.4	3.1	1.4	39.0	37.0	30.0	39.0	36.3	4.3	2.1
16.06.94	9	35.0	31.0	37.0	37.0	29.0	33.8	3.6	1.6	38.0	38.0	31.0	37.0	36.0	3.4	1.7
23.06.94	10	29.0	31.0	37.0	37.0	28.0	32.4	4.3	1.9	38.0	38.0	30.0	37.0	35.8	3.9	1.9
30.06.94	11	31.0	30.0	37.0	39.0	26.0	32.6	5.3	2.4	41.0	38.0	32.0	35.0	36.5	3.9	1.9
07.07.94	12	30.0	33.0	36.0	33.0	26.0	31.6	3.8	1.7	39.0	38.0	31.0	37.0	36.3	3.6	1.8
14.07.94	13	26.0	29.0	29.0	37.0	23.0	28.8	5.2	2.3	37.0	35.0	30.0	35.0	34.3	3.0	1.5
21.07.94	14	26.0	28.0	32.0	33.0	24.0	28.6	3.9	1.7	36.0	31.0	30.0	35.0	33.0	2.9	1.5
28.07.94	15	27.0	26.0	25.0	26.0	27.0	26.2	0.8	0.4	36.0	37.0	31.0	35.0	34.8	2.6	1.3
04.08.94	16	27.0	26.0		25.0	26.0	26.0	0.8	0.4	38.0	35.0	32.0	33.0	34.5	2.7	1.3
11.08.94	17	27.0	27.0		26.0	23.0	25.8	1.9	0.9	37.0	36.0	31.0	38.0	35.5	3.1	1.6
18.08.94	18	25.0	28.0		27.0	27.0	26.8	1.3	0.6	41.0	36.0	34.0	37.0	37.0	2.9	1.5
25.08.94	19	21.0	30.0		34.0	25.0	27.5	5.7	2.8	41.0	37.0	34.0	38.0	37.5	2.9	1.4
01.09.94	20	14.0	30.0		35.0	24.0	25.8	9.0	4.5	40.0	35.0	32.0	38.0	36.3	3.5	1.8
08.09.94	21	20.0	26.0		35.0	25.0	26.5	6.2	3.1	38.0	33.0	30.0	37.0	34.5	3.7	1.9
15.09.94	22	23.0	33.0		34.0	23.0	28.3	6.1	3.0	40.0	32.0	29.0	35.0	34.0	4.7	2.4
22.09.94	23	29.0	33.0		35.0	24.0	30.3	4.9	2.4	40.0	32.0	30.0	37.0	34.8	4.6	2.3
29.09.94	24	24.0	29.0		35.0	26.0	28.5	4.8	2.4	40.0	32.0	31.0	35.0	34.5	4.0	2.0
06.10.94.	25	30.0	32.0		34.0	23.0	29.8	4.8	2.4	41.0	34.0	33.0	37.0	36.3	3.6	1.8
13.10.94	26				32.0	21.0	26.5	7.8	5.5			33.0	36.0	34.5	2.1	1.5

Appendix Table 4.14

Haemoglobin (Hb) in g/dl.

		Infected group								Uninfected group						
Date	WPI	SH11	SH12	SH13	SH14	SH15	Mean (+)	StDev	SEM	SH.16	SH.17	SH.18	SH.19	Mean (-)	StDev	SEM
31.03.94	-2	14.9	14.3	16.0	13.0	10.5	13.7	2.1	0.9	14.2	12.8	9.6	13.3	12.5	2.0	1.0
07.04.94	-1	16.2	15.1	17.0	14.2	11.7	14.8	2.1	0.9	15.7	13.6	10.4	15.4	13.8	2.4	1.2
14.04.94	0	12.4	14.2	16.8	15.4	10.7	13.9	2.4	1.1	16.3	15.2	11.0	15.7	14.6	2.4	1.2
21.04.94	1	15.0	15.8	16.7	15.3	12.3	15.0	1.7	0.7	15.7	16.4	13.1	15.0	15.1	1.4	0.7
28.04.94	2	15.2	15.4	16.3	15.5	11.3	14.7	2.0	0.9	16.7	16.2	12.3	15.7	15.2	2.0	1.0
05.05.94	3	16.5	16.7	17.1	13.8	11.7	15.2	2.3	1.0	17.0	17.3	12.4	15.0	15.4	2.3	1.1
12.05.94	4	13.2	12.8	12.9	12.9	9.4	12.2	1.6	0.7	14.3	13.4	9.9	12.5	12.5	1.9	0.9
19.05.94	5	17.7	16.0	16.7	15.9	13.7	16.0	1.5	0.7	17.5	17.1	13.1	14.7	15.6	2.1	1.0
26.05.94	6	15.4	15.7	15.7	15.4	12.2	14.9	1.5	0.7	17.5	16.5	12.8	16.2	15.8	2.0	1.0
02.06.94.	7	13.2	16.8	17.7	15.9	13.6	15.4	2.0	0.9	17.9	15.3	13.4	14.9	15.4	1.9	0.9
09.06.94	8	14.9	14.9	16.3	13.5	12.6	14.4	1.4	0.6	16.1	16.3	13.8	16.7	15.7	1.3	0.7
16.06.94	9	13.7	13.8	16.5	16.0	13.2	14.6	1.5	0.7	17.1	16.4	13.2	17.1	16.0	1.9	0.9
23.06.94	10	13.7	14.9	17.3	16.9	12.8	15.1	2.0	0.9	17.0	17.0	13.8	17.4	16.3	1.7	0.8
30.06.94	11	14.0	15.2	16.3	17.1	12.8	15.1	1.7	0.8	18.0	15.9	13.2	16.1	15.8	2.0	1.0
07.07.94	12	13.3	15.6	16.8	16.2	10.8	14.5	2.5	1.1	18.1	17.0	15.5	15.3	16.5	1.3	0.7
14.07.94	13	11.3	13.7	13.0	15.8	10.3	12.8	2.1	1.0	15.5	14.9	12.2	15.2	14.5	1.5	0.8
21.07.94	14	12.2	14.2	13.9	13.5	11.2	13.0	1.3	0.6	15.0	15.9	14.7	16.8	15.6	0.9	0.5
28.07.94	15	12.3	12.1	12.1	11.8	12.4	12.1	0.2	0.1	16.8	16.2	14.9	17.0	16.2	0.9	0.5
04.08.94	16	10.7	12.8		11.0	11.2	11.4	0.9	0.5	15.4	15.0	12.9	15.3	14.7	1.2	0.6
11.08.94	17	11.9	11.7		12.9	11.8	12.1	0.6	0.3	16.8	15.8	13.0	15.2	15.2	1.6	0.8
18.08.94	18	11.0	10.1		12.5	11.7	11.3	1.0	0.5	13.2	15.4	14.3	15.1	14.5	1.0	0.5
25.08.94	19	7.2	12.9		10.9	11.7	10.7	2.5	1.2	16.5	16.5	16.0	17.0	16.5	0.4	0.2
01.09.94	20	6.8	14.0		14.8	12.0	11.9	3.6	1.8	17.1	14.9	13.5	15.4	15.2	1.5	0.7
08.09.94	21	8.8	14.0		14.3	11.5	12.2	2.6	1.3	17.0	13.7	12.6	15.6	14.7	2.0	1.0
15.09.94	22	10.7	14.2		13.8	9.2	12.0	2.4	1.2	16.9	13.3	12.2	14.6	14.3	2.0	1.0
22.09.94	23	12.1	14.1		14.7	10.4	12.8	2.0	1.0	16.8	15.2	12.0	15.8	15.0	2.1	1.0
29.09.94	24	11.9	12.1		14.3	11.8	12.5	1.2	0.6	17.0	14.2	12.4	15.0	14.7	1.9	1.0
06.10.94.	25	13.3	12.9		14.1	9.6	12.5	2.0	1.0	17.2	15.1	13.0	15.8	15.3	1.8	0.9
13.10.94	26		0.0		13.2	9.1	7.4	6.8	1.9			13.5	15.1	14.3	1.1	0.8

Appendix Table 4.15

Mean corposcular volume (MCV) in fl

		Infected group								Uninfected group						
Date	WPI	SH. 11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	StDev	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)	StDev	SEM
31.03.94	-2	24.1	28.8	29.8	29.8	26.0	27.7	2.5	1.1	32.3	32.3	31.7	32.3	32.2	0.3	0.2
07.04.94	-1	33.5	32.0	25.2	29.7	24.4	29.0	4.0	1.8	27.1	23.9	24.5	29.5	26.3	2.6	1.3
14.04.94	0	38.0	32.8	28.0	26.3	23.9	29.8	5.6	2.5	27.0	27.8	26.6	28.9	27.6	1.0	0.5
21.04.94	1	36.6	30.4	25.7	25.8	22.1	28.1	5.6	2.5	33.5	29.7	32.9	29.7	31.5	2.0	1.0
28.04.94	2	23.0	24.0	28.0	28.0	22.5	25.1	2.7	1.2	33.0	30.0	22.0	30.0	28.8	4.7	2.4
05.05.94	3	29.0	25.0	30.0	24.0	21.0	25.8	3.7	1.7	30.0	28.0	24.0	27.0	27.3	2.5	1.3
12.05.94	4	28.7	30.4	27.0	26.3	22.1	26.9	3.1	1.4	28.3	28.8	24.1	30.0	27.8	2.6	1.3
19.05.94	5	30.0	34.0	26.6	30.0	26.0	29.3	3.2	1.4	31.8	32.0	27.4	33.0	31.1	2.5	1.2
26.05.94	6	32.0	31.0	27.7	28.0	26.8	29.1	2.3	1.0	30.0	32.0	27.0	30.6	29.9	2.1	1.1
02.06.94.	7	28.6	32.0	28.6	30.8	31.7	30.3	1.6	0.7	31.0	28.4	31.8	37.5	32.2	3.8	1.9
09.06.94	8	28.6	36.7	27.7	27.0	29.7	29.9	3.9	1.8	30.0	26.0	27.6	33.5	29.3	3.3	1.6
16.06.94	9	29.0	35.0	34.0	27.0	28.0	30.6	3.7	1.6	31.0	36.0	27.0	34.0	32.0	3.9	2.0
23.06.94	10	28.0	35.0	33.0	30.0	30.0	31.2	2.8	1.2	35.0	35.0	32.0	33.0	33.8	1.5	0.8
30.06.94	11	30.0	34.0	35.0	32.0	29.0	32.0	2.6	1.1	36.0	35.0	33.0	34.0	34.5	1.3	0.6
07.07.94	12	34.0	37.0	34.0	27.0	34.0	33.2	3.7	1.7	34.0	35.0	30.0	36.0	33.8	2.6	1.3
14.07.94	13	26.0	36.0	32.0	31.0	33.0	31.6	3.7	1.6	35.5	35.0	31.0	34.0	33.9	2.0	1.0
21.07.94	14	29.0	33.0	35.5	32.0	33.0	32.5	2.4	1.1	34.0	31.0	29.0	33.0	31.8	2.2	1.1
28.07.94	15	28.0	37.0	33.0	31.0	33.5	32.5	3.3	1.5	31.0	35.0	30.0	34.0	32.5	2.4	1.2
04.08.94	16	28.0	38.0		37.0	34.0	34.3	4.5	2.3	34.0	34.0	32.0	32.0	33.0	1.2	0.6
11.08.94	17	29.0	38.0		35.0	33.0	33.8	3.8	1.9	34.0	35.0	30.5	33.0	33.1	1.9	1.0
18.08.94	18	28.0	28.0		28.0	32.0	29.0	2.0	1.0	29.5	29.0	27.0	29.0	28.6	1.1	0.6
25.08.94	19	24.0	37.0		36.0	33.0	32.5	5.9	3.0	34.0	35.0	30.0	33.0	33.0	2.2	1.1
01.09.94	20	46.0	39.0		38.0	28.0	37.8	7.4	3.7	34.0	34.0	31.0	34.0	33.3	1.5	0.8
08.09.94	21	49.0	33.0		36.0	35.0	38.3	7.3	3.6	37.0	34.0	30.0	34.0	33.8	2.9	1.4
15.09.94	22	31.0	43.0		37.0	32.0	35.8	5.5	2.8	36.0	35.0	32.0	32.0	33.8	2.1	1.0
22.09.94	23	31.0	43.0		36.0	37.0	36.8	4.9	2.5	36.0	36.0	30.0	35.0	34.3	2.9	1.4
29.09.94	24	26.0	44.0		37.0	36.0	35.8	7.4	3.7	35.5	34.0	30.5	33.0	33.3	2.1	1.1
06.10.94	25	33.0	41.0		34.0	36.0	36.0	3.6	1.8	35.0	34.6	31.0	34.0	33.7	1.8	0.9
13.10.94	26				28.8	34.5	31.7	4.0	2.9			32.0	34.0	33.0	1.4	1.0

Appendix Table 4.16

Mean Corpuscular Haemoglobulin (MCH) in pg.

	Infected group								Uninfected group						
Date	WPI	SH. 11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	StDev	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)	StDev	SEM
31.03.94	-2	10.0	12.5	12.9	11.4	10.4	11.4	1.3	0.6	13.2	13.3	10.5	11.9	12.2	1.3	0.7
07.04.94	-1	14.3	13.1	10.9	11.7	9.8	12.0	1.8	0.8	11.5	11.2	8.8	13.3	11.2	1.8	0.9
14.04.94	0	12.1	14.5	12.0	11.6	10.2	12.1	1.6	0.7	15.6	11.7	10.8	12.3	12.6	2.1	1.1
21.04.94	1	14.5	13.0	11.9	11.6	9.7	12.1	1.8	0.8	13.9	13.2	13.9	12.0	13.3	0.9	0.4
28.04.94	2	10.0	9.8	12.7	11.5	9.0	10.6	1.5	0.7	14.5	12.8	9.0	12.6	12.2	2.3	1.2
05.05.94	3	12.6	11.5	13.0	8.5	9.5	11.0	2.0	0.9	13.3	12.7	10.0	11.3	11.8	1.5	0.7
12.05.94	4	10.5	11.4	10.3	9.0	8.0	9.8	1.3	0.6	10.6	10.6	8.5	10.4	10.0	1.0	0.5
19.05.94	5	15.0	14.0	13.5	12.8	12.0	13.5	1.1	0.5	13.9	14.0	12.8	12.8	13.4	0.7	0.3
26.05.94	6	13.0	13.6	12.8	12.4	10.9	12.5	1.0	0.5	13.5	14.0	11.0	13.0	12.9	1.3	0.7
02.06.94.	7	10.5	14.0	14.5	13.6	14.9	13.5	1.7	0.8	14.0	12.0	13.0	14.0	13.3	1.0	0.5
09.06.94	8	11.5	15.6	13.0	10.0	13.0	12.6	2.1	0.9	12.0	11.5	12.8	14.0	12.6	1.1	0.5
16.06.94	9	11.0	16.0	15.0	12.0	13.0	13.4	2.1	0.9	14.0	16.0	12.0	16.0	14.5	1.9	1.0
23.06.94	10	13.0	17.0	17.0	14.0	14.0	15.0	1.9	0.8	16.0	16.0	15.0	16.0	15.8	0.5	0.3
30.06.94	11	14.0	17.0	15.0	14.0	14.0	14.8	1.3	0.6	16.0	14.0	14.0	15.0	14.8	1.0	0.5
07.07.94	12	15.0	17.0	16.0	11.0	14.0	14.6	2.3	1.0	17.0	16.0	15.0	15.0	15.8	1.0	0.5
14.07.94	13	11.5	17.0	14.5	13.0	15.0	14.2	2.1	0.9	15.0	15.0	13.0	15.0	14.5	1.0	0.5
21.07.94	14	14.0	17.0	15.0	13.0	15.0	14.8	1.5	0.7	15.0	16.0	14.0	16.0	15.3	1.0	0.5
28.07.94	15	13.0	17.0	16.0	14.0	15.0	15.0	1.6	0.7	14.0	15.0	14.0	16.0	14.8	1.0	0.5
04.08.94	16	11.0	19.0		16.0	14.0	15.0	3.4	1.7	14.0	14.5	13.0	15.0	14.1	0.9	0.4
11.08.94	17	13.0	16.0		17.0	17.0	15.8	1.9	0.9	15.0	15.0	13.0	13.0	14.0	1.2	0.6
18.08.94	18	12.0	10.0		13.0	14.0	12.3	1.7	0.9	9.5	12.0	11.0	12.0	11.1	1.2	0.6
25.08.94	19	8.3	16.0		12.0	16.0	13.1	3.7	1.9	14.0	15.0	14.0	15.0	14.5	0.6	0.3
01.09.94	20	22.0	18.0		15.0	14.0	17.3	3.6	1.8	14.0	15.0	13.0	14.0	14.0	0.8	0.4
08.09.94	21	21.0	18.0		15.0	16.0	17.5	2.7	1.3	16.0	14.0	13.0	14.0	14.3	1.3	0.6
15.09.94	22	14.0	18.0		15.0	13.0	15.0	2.2	1.1	15.0	15.0	13.0	13.0	14.0	1.2	0.6
22.09.94	23	13.0	18.0		15.0	16.0	15.5	2.1	1.0	15.0	17.0	12.0	15.0	14.8	2.1	1.0
29.09.94	24	13.0	18.0		15.0	16.0	15.5	2.1	1.0	15.0	15.0	12.0	14.0	14.0	1.4	0.7
06.10.94.	25	14.0	17.0		14.0	15.0	15.0	1.4	0.7	14.8	15.0	12.0	15.0	14.2	1.5	0.7
13.10.94	26				11.9	15.0	13.5	2.2	1.6			13.0	14.0	13.5	0.7	0.5

Appendix Table 4.17

Mean corpuscular Haemoglobulin concentration (MCHC) in g/dl

	Infected group								Uninfected group						
Date	WPI	SH. 11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	StDev	SEM	SH.16	SH. 17	SH. 18	SH. 19	Mean (-)	StDev	SEM
31.03.94	-2	43.8	43.3	40.0	38.2	37.5	40.6	2.9	1.3	40.6	41.3	33.1	36.9	38.0	3.8	1.9
07.04.94	-1	42.6	40.1	43.6	39.4	40.0	41.1	1.8	0.8	42.4	46.9	35.9	45.0	42.6	4.8	2.4
14.04.94	0	31.0	44.4	44.2	44.0	42.8	41.3	5.8	2.6	42.9	42.2	40.7	42.4	42.1	0.9	0.5
21.04.94	1	39.0	42.7	46.4	45.0	44.0	43.4	2.8	1.3	41.0	44.0	42.0	40.5	41.9	1.5	0.8
28.04.94	2	44.7	40.5	45.0	41.0	40.0	42.2	2.4	1.1	43.9	42.8	39.7	42.4	42.2	1.8	0.9
05.05.94	3	43.4	45.0	57.0	35.4	45.0	45.2	7.7	3.5	43.6	45.5	41.3	41.7	43.0	1.9	1.0
12.05.94	4	36.6	37.7	38.0	34.0	36.0	36.5	1.6	0.7	37.6	36.0	35.4	34.7	35.9	1.2	0.6
19.05.94	5	50.6	41.0	50.6	44.0	47.0	46.6	4.2	1.9	43.8	43.8	46.8	38.7	43.3	3.4	1.7
26.05.94	6	40.5	43.6	46.0	44.0	40.7	43.0	2.3	1.1	44.5	43.4	40.0	42.6	42.6	1.9	1.0
02.06.94.	7	36.7	44.0	50.7	44.0	46.9	44.5	5.1	2.3	45.9	42.5	41.9	38.0	42.1	3.2	1.6
09.06.94	8	40.0	42.6	46.6	37.5	43.4	42.0	3.5	1.5	41.0	44.0	46.0	42.8	43.5	2.1	1.1
16.06.94	9	39.0	44.5	45.0	43.0	45.5	43.4	2.6	1.2	45.0	43.0	43.0	46.0	44.3	1.5	0.8
23.06.94	10	47.0	48.0	47.0	46.0	46.0	46.8	0.8	0.4	45.0	45.0	46.0	47.0	45.8	1.0	0.5
30.06.94	11	45.0	50.0	44.0	44.0	49.0	46.4	2.9	1.3	44.0	42.0	41.0	46.0	43.3	2.2	1.1
07.07.94	12	44.0	47.0	47.0	49.0	41.5	45.7	3.0	1.3	46.0	45.0	50.0	41.0	45.5	3.7	1.9
14.07.94	13	43.0	47.0	45.0	43.0	45.0	44.6	1.7	0.7	42.0	43.0	41.0	43.0	42.3	1.0	0.5
21.07.94	14	47.0	50.0	43.0	41.0	47.0	45.6	3.6	1.6	43.0	51.0	49.0	48.0	47.8	3.4	1.7
28.07.94	15	45.5	46.5	48.0	45.0	46.0	46.2	1.2	0.5	47.0	44.0	48.0	49.0	47.0	2.2	1.1
04.08.94	16	40.0	49.0		44.0	41.0	43.5	4.0	2.0	41.0	43.0	40.0	46.0	42.5	2.7	1.3
11.08.94	17	44.0	43.0		50.0	51.0	47.0	4.1	2.0	45.0	44.0	42.0	40.0	42.8	2.2	1.1
18.08.94	18	44.0	36.0		46.0	43.0	42.3	4.4	2.2	32.0	43.0	41.0	41.0	39.3	4.9	2.5
25.08.94	19	34.0	43.0		32.0	47.0	39.0	7.2	3.6	40.0	45.0	47.0	45.0	44.3	3.0	1.5
01.09.94	20	2.3	47.0		42.0	50.0	35.3	22.3	11.1	43.0	42.0	42.0	41.0	42.0	0.8	0.4
08.09.94	21	40.0	54.0		41.0	46.0	45.3	6.4	3.2	45.0	41.0	42.0	42.0	42.5	1.7	0.9
15.09.94	22	47.0	43.0		41.0	40.0	42.8	3.1	1.6	42.0	42.0	42.0	42.0	42.0	0.0	0.0
22.09.94	23	42.0	43.0		42.0	43.0	42.5	0.6	0.3	42.0	47.5	40.0	43.0	43.1	3.2	1.6
29.09.94	24	50.0	42.0		41.0	45.0	44.5	4.0	2.0	42.5	45.0	40.0	45.0	43.1	2.4	1.2
06.10.94.	25	44.0	40.0		41.0	41.0	41.5	1.7	0.9	42.0	44.0	39.0	43.0	42.0	2.2	1.1
13.10.94	26				41.0	43.0	42.0	1.4	1.0			41.0	42.0	41.5	0.7	0.5

Appendix Table 4.18

Total White Blood cell counts ($x10^9$/ml.)

	Infefected group								Uninfected control						
Date	WPI	SH. 11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	StDev	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)	StDev	SEM
31.03.94	-2	6.600	5.400	5.400	4.840	5.400	5.396	2.92	1.31	9.370	9.340	5.400	4.840	7.238	2.46	1.23
07.04.94	-1	6.220	4.640	6.440	5.040	4.640	5.528	1.695	0.758	7.520	6.060	4.640	5.040	5.815	1.284	0.642
14.04.94	0	5.950	6.600	4.680	6.440	5.040	5.396	1.529	0.684	5.100	6.430	4.680	4.770	5.245	0.81	0.405
21.04.94	1	6.440	12.341	6.337	8.605	11.251	5.742	2.74	1.23	7.584	5.077	4.285	6.349	5.824	3.5	1.75
28.04.94	2	11.811	9.858	9.079	9.810	11.199	8.995	1.119	0.5	5.840	5.794	6.054	4.254	5.486	0.829	0.414
05.05.94	3	16.840	9.002	13.230	15.015	13.748	10.351	2.91	1.3	5.983	7.329	6.425	4.570	6.077	1.15	0.575
12.05.94	4	13.900	11.900	15.200	22.100	11.700	13.567	4.25	1.9	6.800	6.800	7.200	6.700	6.875	0.222	0.111
19.05.94	5	12.941	11.415	11.664	15.631	12.118	14.960	1.71	0.765	6.986	6.687	7.951	5.078	6.676	1.194	0.597
26.05.94	6	11.876	11.911	7.232	10.375	13.098	12.754	2.27	1.01	6.297	6.469	5.408	5.062	5.809	0.681	0.341
02.06.94	7	11.893	10.868	7.490	9.926	13.324	10.898	2.193	0.981	6.242	6.089	6.356	5.360	6.012	0.448	0.224
09.06.94	8	10.884	8.136	11.469	11.328	13.743	10.700	2	0.895	5.893	7.516	5.827	5.220	6.114	0.983	0.491
16.06.94	9	10.203	8.597	10.812	9.488	15.638	11.112	2.75	1.23	6.253	7.114	5.919	4.913	6.050	0.91	0.455
23.06.94	10	9.403	8.894	7.803	8.593	17.136	10.948	3.83	1.71	6.038	6.915	5.329	4.921	5.801	0.875	0.437
30.06.94	11	9.177	9.645	6.546	9.275	17.768	10.366	4.26	1.9	6.291	6.185	5.825	5.347	5.912	0.426	0.213
07.07.94	12	8.165	8.225	8.371	8.756	14.627	10.482	2.8	1.25	5.514	5.916	5.486	5.133	5.512	0.32	0.16
14.07.94	13	9.344	8.424	4.941	8.774	12.450	9.629	2.68	1.2	5.549	5.328	4.965	4.587	5.107	0.422	0.211
21.07.94	14	8.330	8.388	10.083	10.355	9.410	8.787	0.937	0.419	5.230	5.348	5.232	4.131	4.985	0.572	0.286
28.07.94	15	7.088	7.338	17.865	12.204	9.446	9.313	4.46	1.99	5.629	5.807	4.542	4.427	5.101	0.717	0.359
04.08.94	16	8.575	6.452		13.941	8.018	10.788	3.26	1.63	5.306	5.453	5.315	4.386	5.115	0.491	0.245
11.08.94	17	7.675	6.330		7.456	7.739	9.247	0.658	0.329	5.986	6.903	4.829	4.453	5.543	1.117	0.559
18.08.94	18	9.732	6.581		6.974	7.467	7.300	1.41	0.705	6.543	6.134	5.472	4.192	5.585	1.028	0.514
25.08.94	19	10.554	5.312		5.986	6.751	7.689	2.34	1.17	6.084	6.865	5.358	3.998	5.576	1.219	0.609
01.09.94	20	12.287	5.310		5.922	6.232	7.151	3.26	1.63	5.915	5.286	5.641	4.541	5.346	0.595	0.298
08.09.94	21	4.789	4.167		5.166	6.350	7.438	0.919	0.459	5.633	5.370	4.896	4.610	5.127	0.46	0.23
15.09.94	22	4.779	4.741		5.496	6.122	5.118	0.658	0.329	5.543	6.259	4.575	4.220	5.149	0.927	0.464
22.09.94	23	5.465	4.099		5.204	5.294	5.285	0.621	0.31	5.696	5.493	4.643	4.012	4.961	0.78	0.39
29.09.94	24	5.856	3.638		5.233	5.295	5.016	0.954	0.477	5.282	5.417	4.131	3.738	4.642	0.834	0.417
06.10.94	25	5.754	4.382		5.620	10.772	5.006	2.83	1.41	5.675	5.404	4.721	3.931	4.933	0.779	0.39
13.10.94	26				5.802	4.944	6.632	0.607	0.429			4.540	3.795	4.168	0.527	0.372

Appendix Table 4.19

Eosinophil counts ($x10^9$/ml.)

	Infefected group								Uninfected control						
Date	WPI	SH. 11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	StDev	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)	StDev	SEM
31.03.94	-2	0.381	0.119	0.075	0.081	0.025	0.136	0.141	0.063	0.013	0.213	0.056	0.031	0.078	0.092	0.046
07.04.94	-1	0.306	0.162	0.375	0.275	0.206	0.265	0.084	0.037	0.162	0.119	0.219	0.388	0.222	0.118	0.059
14.04.94	1	0.350	0.500	0.338	0.069	0.094	0.270	0.184	0.082	0.044	0.056	0.044	0.294	0.110	0.123	0.062
21.04.94	2	1.756	1.056	1.556	0.663	1.144	1.235	0.431	0.193	0.050	0.313	0.206	0.469	0.260	0.177	0.088
28.04.94	3	6.363	2.825	4.863	2.225	3.131	3.881	1.699	0.760	0.064	0.300	0.262	0.412	0.260	0.145	0.073
05.05.94	4	6.625	4.713	9.350	7.125	3.156	6.190	2.370	1.060	0.144	0.188	0.231	0.081	0.161	0.064	0.032
12.05.94	5	7.519	5.119	8.888	12.550	1.494	7.110	4.140	1.850	0.094	0.119	1.213	0.419	0.461	0.522	0.261
19.05.94	6	7.106	5.850	6.456	7.156	1.581	5.630	2.330	1.040	0.269	0.156	0.625	0.331	0.345	0.200	0.100
26.05.94	7	7.262	7.206	3.694	3.338	3.225	4.945	2.097	0.938	0.106	0.063	0.381	0.356	0.227	0.165	0.083
02.06.94	8	7.769	7.388	4.106	2.575	5.431	5.454	2.191	0.980	0.106	0.088	0.838	0.594	0.406	0.371	0.186
09.06.94	9	6.275	3.125	7.700	2.238	4.906	4.849	2.234	0.999	0.069	0.144	0.419	0.169	0.200	0.152	0.076
16.06.94	10	5.263	2.875	6.038	1.794	7.056	4.605	2.202	0.985	0.113	0.200	0.600	0.088	0.250	0.238	0.119
23.06.94	11	5.331	4.425	3.113	1.606	9.400	4.780	2.940	1.320	0.056	0.169	0.444	0.194	0.216	0.164	0.082
30.06.94	12	4.269	3.763	2.350	2.188	7.875	4.090	2.300	1.030	0.213	0.131	0.475	0.088	0.227	0.173	0.087
07.07.94	13	4.539	3.056	2.339	2.450	6.162	3.709	1.627	0.728	0.075	0.056	0.400	0.038	0.142	0.173	0.086
14.07.94	14	4.569	3.381	0.006	2.744	5.656	3.271	2.140	0.957	0.018	0.056	0.244	0.088	0.102	0.099	0.050
21.07.94	15	3.675	3.356	0.000	4.750	1.819	2.720	1.847	0.826	0.006	0.088	0.169	0.056	0.080	0.068	0.034
28.07.94	16	3.506	3.688	0.044	5.081	0.988	2.661	2.078	0.929	0.081	0.088	0.225	0.169	0.141	0.069	0.035
04.08.94	17	3.306	1.875		2.063	0.506	1.938	1.146	0.573	0.038	0.025	0.131	0.063	0.064	0.047	0.024
11.08.94	18	2.913	1.244		0.356	0.306	1.205	1.218	0.609	0.162	0.225	0.175	0.113	0.169	0.046	0.023
18.08.94	19	3.325	0.850		0.119	0.319	1.153	1.480	0.740	0.156	0.094	0.181	0.125	0.139	0.038	0.019
25.08.94	20	3.262	0.756		0.175	0.419	1.153	1.426	0.713	0.113	0.044	0.144	0.050	0.088	0.049	0.024
01.09.94	21	1.919	0.387		0.100	0.081	0.622	0.876	0.438	0.131	0.075	0.319	0.081	0.152	0.115	0.057
08.09.94	22	0.700	0.275		0.144	0.312	0.358	0.239	0.120	0.119	0.075	0.138	0.094	0.107	0.028	0.014
15.09.94	23	0.463	0.238		0.150	0.113	0.241	0.157	0.079	0.100	0.081	0.225	0.144	0.138	0.064	0.032
22.09.94	24	0.788	0.213		0.100	0.075	0.294	0.335	0.167	0.050	0.031	0.181	0.081	0.086	0.067	0.033
29.09.94	25	1.069	0.288		0.119	0.156	0.408	0.447	0.223	0.069	0.019	0.194	0.113	0.099	0.074	0.037
06.10.94	26	1.106	0.375		0.119	0.069	0.417	0.478	0.239	0.119	0.000	0.075	0.056	0.063	0.049	0.025
13.10.94	27				0.175	0.056	0.116	0.084	0.060			0.150	0.038	0.094	0.079	0.056

Appendix Table 4.20

Neutrophils diferential counts ($x10^9$/ml.)

		Infefected group								Uninfected control						
Date	WPI	SH. 11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	StDev	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)	StDev	SEM
31.03.94	-2	2.640	4.216	1.731	2.163	1.848	2.52	1.011	0.452	2.249	1.401	0.810	0.629	1.272	0.73	0.365
07.04.94	-1	1.057	1.865	1.128	1.718	0.278	1.209	0.63	0.282	1.504	0.545	0.974	0.454	0.869	0.48	0.24
14.04.94	0	1.785	1.378	1.172	2.295	1.332	1.592	0.453	0.203	0.561	0.836	0.889	0.620	0.7265	0.16	0.08
21.04.94	1	1.159	2.592	0.951	1.721	1.238	1.532	0.656	0.293	1.213	2.318	2.857	0.889	1.819	0.924	0.462
28.04.94	2	1.772	2.070	1.453	1.373	1.008	1.535	0.404	0.181	1.226	1.564	1.453	0.553	1.199	0.453	0.227
05.05.94	3	1.347	0.090	0.794	0.450	2.337	1.004	0.878	0.392	1.376	1.099	1.478	0.594	1.137	0.396	0.198
12.05.94	4	1.390	1.666	2.128	2.210	0.936	1.666	0.529	0.236	1.564	1.360	1.152	0.938	1.253	0.269	0.135
19.05.94	5	3.235	1.484	1.750	2.032	0.242	1.749	1.076	0.481	1.327	0.736	4.373	1.117	1.888	1.674	0.837
26.05.94	6	1.663	1.072	0.651	1.141	2.227	1.351	0.608	0.272	0.882	1.747	1.298	0.810	1.184	0.432	0.216
02.06.94.	7	0.951	1.522	0.824	1.191	1.732	1.244	0.381	0.17	1.061	1.218	1.335	0.590	1.051	0.327	0.164
09.06.94	8	1.197	1.627	1.262	1.812	1.374	1.454	0.259	0.116	1.179	1.954	2.389	1.357	1.72	0.556	0.278
16.06.94	9	1.530	2.149	1.081	1.423	1.720	1.581	0.394	0.176	1.126	0.996	1.184	0.933	1.0598	0.115	0.058
23.06.94	10	1.410	1.512	0.936	1.633	0.857	1.27	0.351	0.157	1.268	1.245	1.013	0.984	1.1275	0.15	0.075
30.06.94	11	1.744	2.025	0.851	1.855	3.554	2.006	0.978	0.437	1.384	1.794	1.456	1.123	1.439	0.276	0.138
07.07.94	12	1.143	1.316	1.088	1.751	3.803	1.82	1.139	0.509	0.937	1.183	1.152	0.975	1.0617	0.124	0.062
14.07.94	13	1.215	1.601	3.014	1.228	2.615	1.935	0.83	0.371	0.999	0.693	0.894	0.963	0.8873	0.137	0.068
21.07.94	14	1.083	1.426	5.949	2.071	1.035	2.313	2.074	0.928	1.046	0.909	1.046	0.289	0.822	0.361	0.181
28.07.94	15	1.134	1.027	13.756	2.563	0.945	3.88	5.56	2.49	1.351	1.161	1.136	0.620	1.067	0.313	0.157
04.08.94	16	1.801	1.548		6.134	0.481	2.49	2.5	1.25	1.061	0.491	1.222	1.009	0.946	0.316	0.158
11.08.94	17	1.305	1.266		1.939	0.619	1.282	0.539	0.27	1.676	1.174	1.207	0.846	1.226	0.342	0.171
18.08.94	18	2.920	0.790		1.813	0.373	1.474	1.138	0.569	1.047	1.104	1.149	1.258	1.1395	0.089	0.045
25.08.94	19	4.433	0.903		0.898	0.338	1.643	1.879	0.939	1.156	1.098	1.072	0.720	1.0115	0.198	0.099
01.09.94	20	7.004	0.956		1.244	0.374	2.39	3.09	1.55	1.360	0.951	1.749	0.727	1.197	0.452	0.226
08.09.94	21	0.814	1.083		1.240	0.953	1.0225	0.182	0.091	1.352	1.235	1.420	2.121	1.532	0.4	0.2
15.09.94	22	1.051	1.327		1.429	1.041	1.212	0.196	0.098	0.887	1.440	1.281	1.055	1.166	0.244	0.122
22.09.94	23	1.148	1.271		1.145	0.741	1.076	0.231	0.116	1.538	1.318	1.486	0.441	1.196	0.512	0.256
29.09.94	24	1.113	0.910		0.890	0.794	0.9268	0.134	0.067	1.056	1.138	1.239	0.635	1.017	0.265	0.133
06.10.94.	25	0.978	1.139		1.349	7.110	2.64	2.98	1.49	1.930	1.135	1.416	0.708	1.297	0.513	0.256
13.10.94	26				1.857	1.335	1.596	0.369	0.261			1.226	1.025	1.125	0.142	0.1

Appendix Table 4.21

Lymphocytes diferential counts ($x10^9$/ml.)

		Infefected group								Uninfected control						
Date	WPI	SH. 11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	StDev	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)	StDev	SEM
31.03.94	-2	3.234	6.323	2.651	6.386	8.085	5.34	2.31	1.03	5.809	6.164	4.320	3.436	4.932	1.278	0.639
07.04.94	-1	4.478	4.817	5.320	8.270	8.250	6.227	1.88	0.841	5.715	5.090	3.202	3.780	4.447	1.157	0.579
14.04.94	0	3.808	3.731	3.794	6.610	5.624	4.713	1.328	0.594	4.080	5.337	3.650	3.244	4.078	0.906	0.453
21.04.94	1	3.606	7.775	3.612	6.454	9.226	6.13	2.51	1.12	5.916	6.953	10.142	4.508	6.88	2.39	1.2
28.04.94	2	2.480	3.943	2.905	6.573	7.503	4.68	2.24	1	4.380	3.650	3.814	2.893	3.684	0.613	0.307
05.05.94	3	3.705	4.321	3.837	6.006	8.799	5.334	2.144	0.959	4.248	5.717	3.919	3.245	4.282	1.044	0.522
12.05.94	4	3.892	5.831	4.256	8.398	9.126	6.3	2.38	1.06	4.488	5.304	4.824	4.489	4.776	0.386	0.193
19.05.94	5	4.012	4.566	3.266	6.721	10.421	5.8	2.89	1.29	5.030	5.751	3.101	3.199	4.27	1.327	0.664
26.05.94	6	2.731	4.764	3.761	6.744	7.990	5.198	2.152	0.962	5.101	4.399	3.623	3.138	4.065	0.864	0.432
02.06.94.	7	4.876	3.478	3.595	6.948	8.394	5.458	2.155	0.964	4.806	4.445	4.322	4.074	4.412	0.305	0.152
09.06.94	8	3.809	3.824	3.555	7.250	7.834	5.254	2.101	0.94	4.361	5.111	2.564	3.445	3.87	1.106	0.553
16.06.94	9	3.775	3.697	4.865	6.262	8.757	5.471	2.11	0.944	4.877	5.264	4.321	3.488	4.488	0.771	0.385
23.06.94	10	3.761	3.113	3.902	5.500	9.768	5.21	2.7	1.21	4.468	5.463	4.050	3.691	4.418	0.766	0.383
30.06.94	11	2.661	4.147	3.011	4.916	4.442	3.835	0.961	0.43	4.592	4.206	4.078	3.689	4.141	0.372	0.186
07.07.94	12	3.184	3.455	3.767	5.166	5.997	4.314	1.212	0.542	4.301	4.260	3.785	3.952	4.075	0.248	0.124
14.07.94	13	3.831	3.454	1.828	4.913	6.350	4.075	1.686	0.754	4.162	4.316	3.624	3.257	3.84	0.489	0.244
21.07.94	14	3.082	2.013	3.327	4.038	6.305	3.753	1.601	0.716	3.975	4.171	3.872	3.635	3.913	0.223	0.112
28.07.94	15	3.119	2.495	2.858	4.515	6.990	3.995	1.841	0.823	4.109	4.355	3.270	3.586	3.83	0.492	0.246
04.08.94	16	4.030	3.420		4.461	6.976	4.722	1.562	0.781	3.873	4.853	3.827	3.290	3.961	0.651	0.326
11.08.94	17	4.298	3.735		5.145	6.501	4.92	1.203	0.601	4.250	5.522	3.429	3.340	4.135	1.011	0.506
18.08.94	18	3.601	4.607		4.882	6.496	4.897	1.2	0.6	5.234	4.907	3.776	2.809	4.181	1.108	0.554
25.08.94	19	2.955	2.762		4.609	6.008	4.083	1.527	0.764	4.746	5.286	3.965	3.038	4.259	0.978	0.489
01.09.94	20	3.317	3.770		4.205	5.609	4.225	0.991	0.496	4.141	4.229	3.610	3.360	3.835	0.418	0.209
08.09.94	21	2.682	2.709		3.668	5.144	3.551	1.157	0.578	4.112	3.598	3.182	2.351	3.311	0.744	0.372
15.09.94	22	3.011	3.034		3.627	4.836	3.627	0.855	0.427	4.324	4.694	2.791	3.038	3.712	0.938	0.469
22.09.94	23	3.170	2.623		3.435	4.288	3.379	0.694	0.347	4.044	4.010	2.972	3.571	3.649	0.5	0.25
29.09.94	24	3.338	2.437		3.715	4.130	3.405	0.722	0.361	4.067	4.225	2.520	2.990	3.451	0.828	0.414
06.10.94.	25	3.395	2.804		4.046	3.662	3.379	0.522	0.261	3.405	3.837	3.021	3.105	3.342	0.369	0.184
13.10.94	26				3.597	3.362	3.477	0.166	0.117			2.860	2.467	2.663	0.278	0.196

Appendix Table 4.22

Monocytes diferential counts ($x10^9$/ml.)

		Infefected group								Uninfected control						
Date	WPI	SH. 11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	StDev	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)	StDev	SEM
31.03.94	-2	0.264	0.117	0.108	0.515	0.578	0.3164	0.22	0.098	0.562	0.374	0.162	0.484	0.3955	0.174	0.087
07.04.94	-1	0.062	0.155	0.484	0.107	0.371	0.2358	0.183	0.082	0.226	0.242	0.000	0.151	0.1547	0.111	0.055
14.04.94	0	0.060	0.115	0.335	0.184	0.222	0.1832	0.105	0.047	0.255	0.129	0.047	0.143	0.1435	0.086	0.043
21.04.94	1	0.322	0.000	0.380	0.172	0.338	0.2424	0.157	0.07	0.152	0.403	0.429	0.063	0.2617	0.182	0.091
28.04.94	2	0.472	0.197	0.182	0.000	0.448	0.2598	0.199	0.089	0.058	0.348	0.182	0.085	0.1682	0.131	0.066
05.05.94	3	0.337	0.090	0.132	0.450	0.275	0.2568	0.148	0.066	0.120	0.147	0.257	0.091	0.1538	0.073	0.036
12.05.94	4	0.278	0.119	0.152	0.221	0.234	0.2008	0.064	0.029	0.476	0.068	0.144	0.268	0.239	0.178	0.089
19.05.94	5	0.259	0.114	0.233	0.469	0.485	0.312	0.16	0.072	0.349	0.134	0.080	0.051	0.1535	0.135	0.067
26.05.94	6	0.238	0.000	0.289	0.519	0.262	0.2616	0.184	0.083	0.063	0.259	0.108	0.101	0.1328	0.087	0.043
02.06.94.	7	0.119	0.109	0.000	0.496	0.533	0.251	0.245	0.11	0.062	0.426	0.127	0.322	0.2342	0.169	0.085
09.06.94	8	0.000	0.244	0.115	0.453	0.550	0.272	0.229	0.102	0.118	0.150	0.117	0.052	0.1093	0.041	0.021
16.06.94	9	0.102	0.086	0.216	0.095	0.313	0.1624	0.1	0.045	0.125	0.569	0.118	0.246	0.264	0.211	0.106
23.06.94	10	0.188	0.089	0.000	0.000	0.000	0.0554	0.084	0.037	0.181	0.000	0.000	0.049	0.0575	0.086	0.043
30.06.94	11	0.092	0.386	0.000	0.093	0.355	0.1852	0.174	0.078	0.126	0.124	0.058	0.053	0.0903	0.04	0.02
07.07.94	12	0.082	0.000	0.251	0.000	0.146	0.0958	0.106	0.048	0.110	0.237	0.219	0.103	0.1673	0.071	0.035
14.07.94	13	0.280	0.084	0.049	0.000	0.000	0.0826	0.116	0.052	0.055	0.266	0.099	0.000	0.105	0.115	0.057
21.07.94	14	0.000	0.084	0.706	0.104	0.094	0.198	0.287	0.128	0.105	0.267	0.052	0.041	0.1162	0.104	0.052
28.07.94	15	0.284	0.147	1.072	0.244	0.189	0.387	0.386	0.173	0.113	0.116	0.136	0.089	0.1135	0.019	0.01
04.08.94	16	0.000	0.000		0.836	0.160	0.249	0.399	0.199	0.212	0.109	0.159	0.000	0.12	0.09	0.045
11.08.94	17	0.077	0.063		0.075	0.387	0.1505	0.158	0.079	0.060	0.000	0.048	0.045	0.0382	0.026	0.013
18.08.94	18	0.292	0.132		0.139	0.224	0.1968	0.076	0.038	0.065	0.061	0.055	0.042	0.05575	0.01	0.005
25.08.94	19	0.106	0.053		0.120	0.135	0.1035	0.036	0.018	0.183	0.412	0.054	0.120	0.1922	0.156	0.078
01.09.94	20	0.246	0.212		0.178	0.187	0.2058	0.03	0.015	0.118	0.106	0.113	0.091	0.107	0.012	0.006
08.09.94	21	0.096	0.167		0.103	0.000	0.0915	0.069	0.034	0.169	0.483	0.098	0.046	0.199	0.196	0.098
15.09.94	22	0.287	0.047		0.220	0.245	0.1998	0.106	0.053	0.166	0.125	0.275	0.042	0.152	0.097	0.048
22.09.94	23	0.055	0.041		0.208	0.053	0.0892	0.079	0.04	0.000	0.110	0.046	0.000	0.039	0.052	0.026
29.09.94	24	0.000	0.036		0.262	0.106	0.101	0.116	0.058	0.106	0.000	0.207	0.037	0.0875	0.091	0.046
06.10.94.	25	0.115	0.000		0.056	0.000	0.0428	0.055	0.028	0.057	0.216	0.094	0.000	0.0918	0.091	0.046
13.10.94	26				0.232	0.148	0.19	0.059	0.042			0.272	0.228	0.25	0.031	0.022

Appendix Table 4.23:

Eosinophil diferential counts ($x10^9$/ml.)

		Infefected group								Uninfected control						
Date	WPI	SH. 11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	StDev	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)	StDev	SEM
31.03.94	-2	0.396	1.054	0.920	1.133	1.040	0.909	0.297	0.133	0.750	1.588	0.108	0.290	0.684	0.66	0.33
07.04.94	-1	0.622	0.855	1.048	0.644	0.371	0.708	0.256	0.115	0.075	0.182	0.464	0.655	0.344	0.264	0.132
14.04.94	0	0.298	0.517	0.279	0.092	0.222	0.2816	0.154	0.069	0.204	0.129	0.094	0.763	0.298	0.314	0.157
21.04.94	1	1.352	1.975	1.394	0.258	0.450	1.086	0.715	0.32	0.303	0.403	0.857	0.889	0.613	0.303	0.152
28.04.94	2	7.087	3.647	4.540	1.864	2.240	3.876	2.093	0.936	0.175	0.232	0.605	0.723	0.434	0.271	0.136
05.05.94	3	11.451	4.501	8.467	8.108	2.337	6.97	3.58	1.6	0.239	0.366	0.771	0.640	0.504	0.244	0.122
12.05.94	4	8.340	4.284	8.664	11.271	1.404	6.79	3.91	1.75	0.272	0.068	1.008	1.005	0.588	0.49	0.245
19.05.94	5	5.176	5.251	6.415	6.409	0.969	4.84	2.25	1.01	0.279	0.067	0.398	0.711	0.364	0.269	0.134
26.05.94	6	7.244	6.075	2.531	1.971	2.620	4.09	2.4	1.07	0.252	0.065	0.379	1.012	0.427	0.411	0.205
02.06.94.	7	5.828	5.760	3.071	1.290	2.665	3.723	2.003	0.896	0.312	0.000	0.572	0.375	0.315	0.237	0.119
09.06.94	8	5.877	2.441	6.308	1.812	3.985	4.085	2.002	0.895	0.236	0.301	0.758	0.365	0.415	0.235	0.117
16.06.94	9	4.795	2.665	4.541	1.708	4.848	3.711	1.437	0.643	0.125	0.285	0.296	0.246	0.238	0.078	0.039
23.06.94	10	4.043	4.180	2.809	1.461	6.683	3.835	1.934	0.865	0.121	0.207	0.266	0.197	0.1978	0.06	0.03
30.06.94	11	4.680	3.086	2.684	2.412	9.417	4.46	2.91	1.3	0.189	0.062	0.233	0.481	0.2412	0.176	0.088
07.07.94	12	3.674	3.455	3.265	1.839	4.681	3.383	1.021	0.457	0.165	0.237	0.274	0.103	0.1947	0.076	0.038
14.07.94	13	4.018	3.285	0.049	2.632	3.486	2.694	1.56	0.697	0.333	0.053	0.348	0.367	0.2752	0.149	0.074
21.07.94	14	4.165	4.781	0.101	3.935	1.976	2.992	1.927	0.862	0.105	0.000	0.209	0.165	0.1198	0.091	0.045
28.07.94	15	2.481	3.449	0.179	4.882	1.322	2.463	1.826	0.817	0.056	0.174	0.000	0.133	0.0908	0.078	0.039
04.08.94	16	2.744	1.484		2.509	0.401	1.785	1.072	0.536	0.159	0.000	0.106	0.088	0.0882	0.066	0.033
11.08.94	17	1.996	1.266		0.298	0.155	0.929	0.866	0.433	0.000	0.207	0.097	0.223	0.1318	0.104	0.052
18.08.94	18	2.920	1.053		0.139	0.373	1.121	1.26	0.63	0.196	0.061	0.492	0.084	0.2083	0.198	0.099
25.08.94	19	3.061	1.594		0.359	0.135	1.287	1.345	0.673	0.000	0.069	0.268	0.120	0.1143	0.114	0.057
01.09.94	20	1.720	0.372		0.296	0.062	0.613	0.75	0.375	0.296	0.000	0.169	0.363	0.207	0.16	0.08
08.09.94	21	1.197	0.208		0.052	0.254	0.428	0.52	0.26	0.000	0.054	0.196	0.092	0.0855	0.083	0.041
15.09.94	22	0.430	0.332		0.220	0.000	0.2455	0.185	0.092	0.166	0.000	0.229	0.084	0.1198	0.1	0.05
22.09.94	23	1.093	0.164		0.416	0.212	0.471	0.429	0.214	0.114	0.055	0.139	0.000	0.077	0.062	0.031
29.09.94	24	1.405	0.255		0.366	0.265	0.573	0.557	0.279	0.053	0.054	0.165	0.075	0.0868	0.053	0.027
06.10.94.	25	1.208	0.438		0.169	0.000	0.454	0.534	0.267	0.284	0.216	0.189	0.118	0.2018	0.069	0.034
13.10.94	26				0.116	0.099	0.1075	0.012	0.009			0.182	0.114	0.148	0.048	0.034

Appendix Table 4.24

Basophils diferential counts ($x10^9$/ml)

	Infefected group								Uninfected control						
Date	WPI	SH. 11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	StDev	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)	StDev	SEM
31.03.94	-2	0.066	0.000	0.000	0.103	0.000	0.034	0.048	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000
07.04.94	-1	0.000	0.000	0.081	0.000	0.000	0.016	0.036	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000
14.04.94	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
21.04.94	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
28.04.94	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
05.05.94	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
12.05.94	4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.072	0.000	0.018	0.036	0.018
19.05.94	5	0.259	0.000	0.000	0.000	0.000	0.052	0.116	0.052	0.000	0.000	0.000	0.000	0.000	0.000	0.000
26.05.94	6	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
02.06.94.	7	0.119	0.000	0.000	0.000	0.000	0.024	0.053	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000
09.06.94	8	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
16.06.94	9	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
23.06.94	10	0.000	0.000	0.156	0.000	0.000	0.031	0.070	0.031	0.000	0.000	0.000	0.000	0.000	0.000	0.000
30.06.94	11	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
07.07.94	12	0.082	0.000	0.000	0.000	0.000	0.016	0.037	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000
14.07.94	13	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
21.07.94	14	0.000	0.084	0.000	0.207	0.000	0.058	0.091	0.041	0.000	0.000	0.052	0.000	0.013	0.026	0.013
28.07.94	15	0.071	0.000	0.000	0.000	0.000	0.014	0.032	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000
04.08.94	16	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
11.08.94	17	0.000	0.000		0.000	0.077	0.019	0.039	0.019	0.000	0.000	0.048	0.000	0.012	0.024	0.012
18.08.94	18	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
25.08.94	19	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
01.09.94	20	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
08.09.94	21	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
15.09.94	22	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
22.09.94	23	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
29.09.94	24	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
06.10.94.	25	0.058	0.000		0.000	0.000	0.015	0.029	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000
13.10.94	26				0.000	0.000	0.000	0.000	0.000			0.000	0.000	0.000	0.000	0.000

Appendix Table 4.25

Albumin (g/l) in sheep

	Infefected group								Uninfected control						
Date	WPI	SH. 11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	StDev	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)	StDev	SEM
31.03.94	-2	48.2	46.5	48.7	36.1	40.6	44.02	5.48	2.45	39.6	39.6	34.2	32.2	36.4	3.78	1.89
07.04.94	-1	35.8	41.3	34.9	37.1	46.4	39.1	4.76	2.13	36.1	37.6	34.0	40.7	37.1	2.82	1.41
14.04.94	0	34.9	38.1	41.7	37.6	41.0	38.66	2.75	1.23	29.9	38.5	33.1	35.9	34.35	3.7	1.85
21.04.94	1	38.6	38.2	41.9	34.0	30.6	36.66	4.40	1.97	41.6	34.4	32.1	36.3	36.1	4.05	2.02
28.04.94	2	35.7	34.6	32.2	40.9	31.4	34.96	3.75	1.68	35.1	45.6	33.9	38.6	38.3	5.26	2.63
05.05.94	3	29.9	32.2	33.7	34.0	37.3	33.42	2.71	1.21	26.9	33.6	34.2	42.8	34.37	6.52	3.26
12.05.94	4	35.8	35.4	35.2	38.5	44.4	37.86	3.89	1.74	45.0	41.3	35.4	38.1	39.95	4.14	2.07
19.05.94	5	38.0	32.4	37.4	31.1	41.6	36.1	4.31	1.93	49.9	45.9	40.6	40.7	44.28	4.49	2.25
26.05.94	6	37.0	48.4	38.3	39.5	39.8	40.6	4.50	2.01	47.8	49.5	31.7	48.5	44.38	8.48	4.24
02.06.94.	7	38.3	40.9	38.0	40.4	39.3	39.38	1.27	0.57	49.3	44.4	43.1	39.7	44.13	3.98	1.99
09.06.94	8	33.4	33.3	33.4	35.7	38.0	34.76	2.07	0.93	39.7	37.3	39.4	36.4	38.2	1.61	0.80
16.06.94	9	37.2	31.0	34.8	39.8	36.0	35.76	3.24	1.45	38.4	39.5	34.4	40.3	38.15	2.62	1.31
23.06.94	10	32.9	35.2	33.9	44.1	33.9	36	4.60	2.06	43.5	38.0	32.1	41.3	38.72	4.96	2.48
30.06.94	11	33.9	31.5	40.5	38.9	33.8	35.72	3.80	1.70	41.6	38.8	37.3	41.7	39.85	2.17	1.08
07.07.94	12	30.3	30.6	35.4	31.3	35.2	32.56	2.53	1.13	43.8	41.4	41.0	37.5	40.92	2.60	1.30
14.07.94	13	31.1	30.1	31.8	34.0	31.0	31.6	1.47	0.66	37.5	34.8	36.3	38.7	36.825	1.67	0.83
21.07.94	14	30.6	30.9	23.0	32.1	28.4	29	3.61	1.61	41.3	37.4	36.3	40.1	38.78	2.32	1.16
28.07.94	15	29.9	28.1	23.6	29.5	29.6	28.14	2.63	1.18	38.7	39.1	35.8	40.3	38.475	1.91	0.95
04.08.94	16	31.3	28.1		28.5	31.6	29.875	1.83	0.92	39.2	38.9	35.6	39.6	38.325	1.84	0.92
11.08.94	17	30.6	28.4		30.4	29.4	29.7	1.01	0.51	39.2	37.0	36.7	39.3	38.05	1.39	0.70
18.08.94	18	27.3	29.6		27.6	32.1	29.15	2.22	1.11	37.4	56.8	35.3	39.8	42.33	9.82	4.91
25.08.94	19	24.9	27.9		33.8	29.3	28.97	3.70	1.85	39.4	39.2	37.9	41.4	39.475	1.45	0.72
01.09.94	20	26.1	28.3		29.0	32.0	28.85	2.44	1.22	38.7	36.8	33.3	35.7	36.12	2.25	1.13
08.09.94	21	27.2	31.8		32.4	31.8	30.8	2.42	1.21	61.8	31.4	34.5	39.6	41.83	13.74	6.87
15.09.94	22	24.4	32.0		32.2	29.0	29.4	3.64	1.82	35.3	34.1	38.5	35.6	35.875	1.87	0.93
22.09.94	23	22.0	21.7		24.1	25.8	23.4	1.92	0.96	30.0	26.9	29.1	34.4	30.1	3.15	1.57
29.09.94	24	24.1	26.0		31.2	30.0	27.83	3.33	1.67	32.8	30.0	32.8	33.4	32.25	1.53	0.76
06.10.94.	25	28.7	29.1		33.7	33.7	31.3	2.78	1.39	40.7	37.5	36.5	35.1	37.45	2.38	1.19
13.10.94	26				28.8	26.1	27.45	1.91	1.35			31.0	40.3	35.65	6.58	4.65

Appendix Table 4.26

Total protein (g/l) in sheep

	Infefected group									Uninfected control						
Date	WPI	SH. 11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	StDev	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)	StDev	SEM
31.03.94	-2	10.0	9.1	8.5	7.6	7.2	8.48	1.13	0.505	7.5	9.3	7.1	7.2	7.8	7.8	1.0
07.04.94	-1	9.2	8.9	7.9	8.0	8.0	8.4	0.604	0.27	8.6	9.2	9.1	8.1	8.8	8.8	0.5
14.04.94	0	8.9	8.0	8.5	8.0	9.1	8.5	0.505	0.226	6.8	8.8	7.0	6.4	7.3	7.3	1.063
21.04.94	1	9.5	8.1	7.3	7.4	7.5	7.96	0.915	0.409	7.2	10.0	7.2	7.3	7.9	7.9	1.384
28.04.94	2	8.6	7.9	8.1	7.9	7.1	7.92	0.54	0.242	6.9	10.9	8.9	7.8	8.6	8.6	1.723
05.05.94	3	9.1	7.7	8.4	7.3	7.0	7.9	0.851	0.381	7.4	8.2	8.0	8.3	8.0	8.0	0.403
12.05.94	4	7.1	7.5	8.5	8.0	7.5	7.72	0.54	0.242	7.5	7.5	8.5	7.5	7.8	7.8	0.5
19.05.94	5	7.7	7.6	10.0	7.4	10.0	8.54	1.337	0.598	7.4	7.6	8.0	7.5	7.6	7.6	0.263
26.05.94	6	8.5	8.3	9.5	7.6	9.2	8.62	0.753	0.337	7.3	6.9	9.1	8.2	7.9	7.9	0.981
02.06.94.	7	8.5	7.3	7.9	8.3	8.9	8.18	0.61	0.273	7.9	7.6	7.6	7.3	7.6	7.6	0.245
09.06.94	8	7.5	7.4	8.0	7.8	9.5	8.04	0.85	0.38	6.9	7.8	8.6	7.2	7.6	7.6	0.75
16.06.94	9	8.2	10.0	8.6	7.9	8.6	8.66	0.805	0.36	7.9	7.8	8.9	7.4	8.0	8.0	0.638
23.06.94	10	8.5	9.2	8.7	7.4	9.1	8.58	0.719	0.322	7.7	8.9	7.2	8.7	8.1	8.1	0.81
30.06.94	11	8.2	8.3	8.4	8.7	10.0	8.72	0.74	0.331	7.6	8.0	6.9	8.6	7.8	7.8	0.714
07.07.94	12	11.8	8.0	7.6	8.9	9.2	9.1	1.643	0.735	7.6	8.5	7.2	7.9	7.8	7.8	0.548
14.07.94	13	10.0	11.0	7.6	8.6	7.8	9	1.463	0.654	7.4	8.0	7.9	8.3	7.9	7.9	0.374
21.07.94	14	9.4	8.5	7.3	8.9	8.3	8.48	0.782	0.35	7.4	9.1	7.8	8.1	8.1	8.1	0.726
28.07.94	15	9.3	8.2	7.2	8.3	7.9	8.18	0.76	0.34	6.9	7.6	7.5	8.2	7.6	7.6	0.532
04.08.94	16	9.2	8.4		7.6	7.8	8.25	0.719	0.359	7.4	8.0	7.4	7.7	7.6	7.6	0.287
11.08.94	17	8.8	7.9		7.6	7.3	7.9	0.648	0.324	7.8	7.7	7.5	8.2	7.8	7.8	0.294
18.08.94	18	10.0	7.6		7.0	8.0	8.2	1.3	0.65	7.4	7.9	7.5	8.2	7.8	7.75	0.37
25.08.94	19	10.9	7.0		7.7	6.6	8.1	1.954	0.977	8.6	7.7	7.6	8.6	8.1	8.125	0.55
01.09.94	20	7.9	8.0		7.7	6.6	7.6	0.645	0.323	8.9	7.8	7.7	8.4	8.2	8.2	0.56
08.09.94	21	7.1	7.1		7.5	5.0	6.7	1.132	0.57	7.9	8.7	7.9	7.8	8.1	8.075	0.419
15.09.94	22	6.4	5.6		5.8	4.9	5.7	0.618	0.31	8.5	8.9	7.6	9.2	8.6	8.55	0.695
22.09.94	23	6.7	5.2		2.8	5.5	5.1	1.634	0.82	8.3	8.6	7.0	9.1	8.3	8.25	0.896
29.09.94	24	4.5	4.4		4.5	4.6	4.5	0.1	0.0	8.4	8.9	9.1	7.4	8.5	8.45	0.759
06.10.94.	25	6.8	5.3		5.6	5.3	5.8	0.7	0.4	8.1	6.5	7.0	7.2	7.2	7.2	0.668
13.10.94	26				6.7	6.1	6.4	0.4	0.3			7.8	7.6	7.7	7.7	0.141

Appendix Table 4.27

Glutamate Dehydrogenase (IU/l) in sheep

	Infefected group									Uninfected control						
Date	WPI	SH. 11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	StDev	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)	StDev	SEM
31.03.94	-2	0.2	1.2	2.2	1.4	8.7	2.7	3.4	1.5	0.2	0.6	1.0	0.6	0.6	0.3	0.2
07.04.94	-1	2.0	0.2	2.4	2.2	11.6	3.7	4.5	2.0	0.4	3.2	3.5	3.5	2.7	1.5	0.8
14.04.94	0	3.2	3.0	4.1	0.4	5.9	3.3	2.0	0.9	-1.0	0.0	1.2	8.3	2.1	4.2	2.1
21.04.94	1	3.0	-1.0	22.1	19.1	3.3	9.3	10.5	4.7	2.4	9.1	2.8	4.9	4.8	3.1	1.5
28.04.94	2	19.7	9.7	25.8	9.3	11.4	15.2	7.3	3.3	1.0	6.3	3.2	9.3	5.0	3.6	1.8
05.05.94	3	87.3	29.4	108.7	12.0	12.0	49.9	45.1	20.2	1.0	2.4	3.3	3.5	2.6	1.1	0.6
12.05.94	4	110.7	53.4	130.2	21.9	13.8	66.0	52.3	23.4	0.6	7.9	4.1	6.3	4.7	3.2	1.6
19.05.94	5	85.3	72.3	98.9	32.1	17.3	61.2	35.0	15.7	0.0	7.1	1.8	9.7	4.7	4.5	2.3
26.05.94	6	81.4	63.0	57.5	28.6	17.7	49.6	26.0	11.6	0.0	9.5	8.7	9.9	7.0	4.7	2.4
02.06.94.	7	80.2	61.9	57.3	45.3	44.9	57.9	14.5	6.5	0.0	4.1	3.9	9.9	4.5	4.1	2.0
09.06.94	8	72.7	37.4	61.3	24.4	22.9	43.7	22.3	10.0	6.3	2.2	6.5	4.1	4.8	2.0	1.0
16.06.94	9	52.4	26.8	40.8	24.4	26.4	34.2	12.1	5.4	6.1	1.2	5.7	1.8	3.7	2.6	1.3
23.06.94	10	43.5	29.3	16.7	11.0	18.1	23.7	12.9	5.8	6.5	6.1	6.3	8.7	6.9	1.2	0.6
30.06.94	11	35.1	38.6	24.8	20.3	32.9	30.3	7.6	3.4	8.0	3.0	5.5	3.9	5.1	2.2	1.1
07.07.94	12	23.8	21.5	18.7	52.0	18.7	26.9	14.2	6.3	7.9	6.1	12.0	6.5	8.1	2.7	1.4
14.07.94	13	33.9	25.0	8.5	29.7	34.3	26.3	10.6	4.8	2.6	15.4	15.2	6.3	9.9	6.4	3.2
21.07.94	14	36.1	73.9	24.4	80.4	54.2	53.8	23.9	10.7	2.8	1.3	0.4	7.5	3.0	3.2	1.6
28.07.94	15	24.8	39.2	8.5	31.5	36.1	28.0	12.2	5.5	7.7	7.2	6.7	3.0	6.2	2.1	1.1
04.08.94	16	19.7	26.6		42.4	36.8	31.4	10.2	5.1	6.1	3.3	7.3	9.7	6.6	2.7	1.3
11.08.94	17	64.2	38.0		10.0	37.8	37.5	22.1	11.1	2.6	4.1	12.2	9.3	7.1	4.5	2.2
18.08.94	18	92.8	12.4		9.2	17.9	33.1	40.0	20.0	2.8	2.2	8.1	9.4	5.6	3.7	1.8
25.08.94	19	73.7	15.6		11.6	34.5	33.8	28.4	14.2	6.7	1.2	6.3	8.2	5.6	3.1	1.5
01.09.94	20	143.0	11.2		7.1	43.9	51.3	63.3	31.7	7.9	6.5	2.8	7.7	6.2	2.4	1.2
08.09.94	21	24.6	13.4		11.2	42.7	23.0	14.4	7.2	7.1	6.9	3.5	9.3	6.7	2.4	1.2
15.09.94	22	45.1	32.1		16.4	45.3	34.7	13.7	6.8	9.5	3.0	11.8	3.0	6.8	4.5	2.3
22.09.94	23	28.8	60.3		46.5	40.6	44.1	13.1	6.6	4.1	2.6	7.9	9.3	6.0	3.1	1.6
29.09.94	24	63.8	31.5		20.0	68.4	45.9	23.8	11.9	2.2	10.4	13.0	9.7	8.8	4.6	2.3
06.10.94.	25	63.8	21.5		15.4	34.1	33.7	21.5	10.8	1.2	12.6	7.7	12.4	8.5	5.4	2.7
13.10.94	26				10.6	24.4	17.5	9.8	6.9			3.0	12.4	7.7	6.7	4.7

Appendix Table 4.28

Gamma Glutamyltransferase(IU/l) in sheep

		Infefected group								Uninfected control						
Date	WPI	SH. 11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	StDev	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)	StDev	SEM
31.03.94	-2	13.9	17.0	19.3	24.7	26.6	20.3	5.3	2.4	22.4	27.0	32.0	19.7	25.3	5.4	2.7
07.04.94	-1	21.2	23.7	27.8	30.9	24.7	25.7	3.8	1.7	7.7	29.0	20.5	30.9	22.0	10.6	5.3
14.04.94	0	23.5	26.2	21.6	37.4	29.7	27.7	6.2	2.8	27.8	25.5	33.6	20.1	26.8	5.6	2.8
21.04.94	1	26.2	27.4	30.5	28.6	27.0	27.9	1.7	0.7	29.0	30.5	22.0	32.4	28.5	4.5	2.3
28.04.94	2	23.0	24.7	25.9	26.2	25.1	25.0	1.3	0.6	28.6	39.8	41.7	29.0	34.8	6.9	3.5
05.05.94	3	30.9	30.1	44.4	34.7	28.6	33.7	6.4	2.9	31.7	39.8	36.3	23.9	32.9	6.9	3.4
12.05.94	4	28.2	23.5	43.2	28.2	25.5	29.7	7.8	3.5	32.8	33.6	27.4	24.7	29.6	4.3	2.1
19.05.94	5	21.6	27.0	46.7	30.9	25.9	30.4	9.7	4.3	30.5	30.5	24.7	21.2	26.7	4.6	2.3
26.05.94	6	31.7	32.0	42.8	35.1	27.8	33.9	5.6	2.5	25.9	30.1	25.5	21.2	25.7	3.6	1.8
02.06.94.	7	44.0	34.0	47.9	30.5	30.5	37.4	8.1	3.6	26.6	33.2	26.6	21.6	27.0	4.8	2.4
09.06.94	8	42.8	30.9	42.5	32.8	33.6	36.5	5.7	2.5	27.8	34.7	25.1	24.3	28.0	4.7	2.4
16.06.94	9	36.3	22.8	31.7	25.1	32.1	29.6	5.5	2.5	22.4	27.4	24.3	21.2	23.8	2.7	1.4
23.06.94	10	43.6	28.6	36.3	29.3	34.4	34.4	6.1	2.7	25.9	28.9	26.2	22.0	25.8	2.8	1.4
30.06.94	11	42.1	30.5	41.7	27.8	56.7	39.8	11.5	5.1	29.3	32.4	27.8	19.3	27.2	5.6	2.8
07.07.94	12	44.8	29.0	35.9	106.0	51.1	53.4	30.6	13.7	38.2	36.4	29.0	21.6	31.3	7.6	3.8
14.07.94	13	40.1	31.3	32.4	43.6	131.6	55.8	42.7	19.1	30.5	31.7	31.7	25.9	30.0	2.8	1.4
21.07.94	14	43.6	61.0	29.0	79.5	98.4	62.3	27.7	12.4	29.3	31.7	32.4	23.9	29.3	3.9	1.9
28.07.94	15	42.8	92.6	32.8	64.5	78.4	62.2	24.7	11.0	27.0	30.9	31.3	23.2	28.1	3.8	1.9
04.08.94	16	28.6	84.5		93.8	45.9	63.2	31.0	15.5	23.9	26.6	27.0	20.0	24.4	3.2	1.6
11.08.94	17	49.0	65.2		61.8	38.2	53.6	12.4	6.2	27.0	26.2	30.9	18.1	25.6	5.4	2.7
18.08.94	18	66.0	52.1		52.9	36.9	52.0	11.9	6.0	24.7	26.6	28.2	20.1	24.9	3.5	1.8
25.08.94	19	90.3	48.3		42.8	31.3	53.2	25.7	12.9	25.5	27.8	29.0	20.1	25.6	3.9	2.0
01.09.94	20	95.7	36.3		39.0	39.8	52.7	28.7	14.4	27.0	28.6	30.9	19.3	26.5	5.0	2.5
08.09.94	21	64.1	29.3		33.6	39.0	41.5	15.6	7.8	25.9	28.6	29.3	20.5	26.1	4.0	2.0
15.09.94	22	59.1	35.1		31.3	33.2	39.7	13.0	6.5	26.6	29.3	31.7	21.6	27.3	4.3	2.2
22.09.94	23	51.3	35.9		27.8	34.0	37.3	10.0	5.0	29.0	30.9	31.7	20.8	28.1	5.0	2.5
29.09.94	24	47.0	43.2		42.8	50.2	45.8	3.5	1.8	28.2	30.1	30.9	20.5	27.4	4.8	2.4
06.10.94.	25	46.3	34.7		23.5	37.1	35.4	9.4	4.7	26.6	29.7	30.5	20.1	26.7	4.7	2.4
13.10.94	26				27.0	29.3	28.2	1.6	1.2			31.3	22.8	27.1	6.0	4.3

Appendix Table 4.29

Serum Glucose levels (mmol/l)

		Infefected group								Uninfected control						
Date	WPI	SH. 11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	StDev	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)	StDev	SEM
31.03.94	-2	2.80	2.80	2.90	2.70	2.25	2.69	0.26	0.11	1.80	2.60	2.40	2.84	2.41	0.45	0.22
07.04.94	-1	2.60	2.30	2.60	2.00	2.04	2.31	0.27	0.12	2.53	2.60	2.00	1.69	2.21	0.44	0.22
14.04.94	0	2.20	2.30	2.20	1.60	2.13	2.09	0.29	0.13	1.82	2.22	2.30	2.22	2.14	0.22	0.11
21.04.94	1	2.30	2.00	2.30	2.30	2.12	2.20	0.16	0.07	2.38	2.01	1.90	2.01	2.08	0.21	0.11
28.04.94	2	2.10	2.10	1.52	1.60	2.36	1.94	0.30	0.13	2.38	2.28	2.40	2.28	2.34	0.06	0.03
05.05.94	3	2.60	1.50	1.68	1.20	1.76	1.75	0.62	0.28	2.10	1.60	2.60	2.13	2.11	0.41	0.20
12.05.94	4	2.20	1.10	1.82	2.60	1.42	1.83	0.60	0.27	2.60	2.84	2.20	2.38	2.51	0.28	0.14
19.05.94	5	2.30	0.60	2.38	2.20	2.60	2.02	0.77	0.34	2.20	1.69	2.30	2.10	2.07	0.27	0.13
26.05.94	6	2.30	2.10	1.82	2.30	2.20	2.14	0.47	0.21	2.30	2.22	2.30	2.60	2.36	0.17	0.08
02.06.94.	7	1.90	1.77	0.40	1.85	2.30	1.64	0.63	0.28	2.38	2.01	1.50	2.20	2.02	0.38	0.19
09.06.94	8	1.50	1.90	1.82	1.50	1.67	1.68	0.18	0.08	2.10	2.28	1.90	2.30	2.15	0.19	0.09
16.06.94	9	1.80	2.10	1.20	1.90	1.20	1.64	0.42	0.19	2.60	2.13	1.60	1.69	2.01	0.46	0.23
23.06.94	10	1.10	1.90	1.40	1.60	1.40	1.48	0.30	0.13	2.20	1.26	1.90	2.09	1.86	0.42	0.21
30.06.94	11	1.40	1.50	1.30	1.50	2.30	1.60	0.17	0.07	2.30	2.60	1.80	2.12	2.21	0.34	0.17
07.07.94	12	2.30	1.80	1.20	1.90	2.38	1.92	0.40	0.18	2.04	2.20	2.00	2.14	2.10	0.09	0.05
14.07.94	13	2.38	2.30	0.50	1.90	2.10	1.84	0.68	0.30	2.38	2.30	2.40	2.13	2.30	0.12	0.06
21.07.94	14	2.10	2.00	0.70	2.10	2.60	1.90	0.48	0.21	2.10	2.30	1.40	1.86	1.92	0.39	0.19
28.07.94	15	2.60	2.00	0.50	2.60	2.20	1.98	0.68	0.31	2.60	2.73	2.00	2.15	2.37	0.35	0.18
04.08.94	16	2.20	2.30		2.80	2.30	2.40	0.57	0.29	2.20	2.03	1.90	2.04	2.04	0.12	0.06
11.08.94	17	2.30	2.38		2.10	2.04	2.21	0.45	0.22	2.30	2.63	2.40	2.61	2.49	0.16	0.08
18.08.94	18	2.04	2.10		2.60	2.41	2.29	0.53	0.27	2.38	2.46	2.38	2.49	2.43	0.06	0.03
25.08.94	19	2.60	2.60		2.80	2.60	2.65	0.33	0.17	2.10	2.73	2.10	1.54	2.12	0.49	0.24
01.09.94	20	2.20	2.20		2.20	2.20	2.20	0.10	0.05	2.60	2.03	2.60	1.81	2.26	0.40	0.20
08.09.94	21	2.30	2.30		2.30	2.30	2.30	0.27	0.14	2.20	2.63	2.20	1.98	2.25	0.27	0.14
15.09.94	22	2.80	2.38		2.30	2.93	2.60	0.18	0.09	2.30	2.46	2.30	2.73	2.45	0.20	0.10
22.09.94	23	2.30	2.10		2.38	2.30	2.27	0.22	0.11	2.21	2.61	2.10	2.03	2.24	0.26	0.13
29.09.94	24	2.30	2.60		2.10	2.38	2.35	0.36	0.18	1.91	2.49	2.60	2.63	2.41	0.34	0.17
06.10.94.	25	2.10	2.10		2.60	2.10	2.23	0.09	0.05	2.21	1.54	2.30	2.46	2.13	0.41	0.20
13.10.94	26				2.20	2.60	2.40	0.06	0.05			2.60	2.18	2.39	0.30	0.21

Appendix Table 4.30

β-hydroxy-butyrate measurements of Sheep

	Infefected group								Uninfected control						
Date	W P I	SH. 11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	StDev	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)	StDev	SEM
31.03.94	-2	0.21	0.25	0.25	0.23	0.20	0.23	0.02	0.01	0.26	0.14	0.17	0.25	0.21	0.06	0.03
07.04.94	-1	0.14	0.17	0.19	0.20	0.18	0.18	0.02	0.01	0.14	0.16	0.23	0.16	0.17	0.04	0.02
14.04.94	0	0.16	0.23	0.23	0.17	0.15	0.19	0.04	0.02	0.19	0.16	0.24	0.27	0.22	0.05	0.02
21.04.94	1	0.16	0.24	0.17	0.26	0.27	0.22	0.05	0.02	0.19	0.19	0.19	0.27	0.21	0.04	0.02
28.04.94	2	0.19	0.19	0.21	0.20	0.15	0.19	0.02	0.01	0.19	0.20	0.15	0.30	0.21	0.06	0.03
05.05.94	3	0.20	0.15	0.23	0.25	0.24	0.21	0.04	0.02	0.22	0.18	0.16	0.31	0.22	0.07	0.03
12.05.94	4	0.18	0.16	0.27	0.24	0.11	0.19	0.06	0.03	0.20	0.25	0.15	0.25	0.21	0.05	0.02
19.05.94	5	0.25	0.15	0.22	0.21	0.29	0.22	0.05	0.02	0.19	0.26	0.16	0.31	0.23	0.07	0.03
26.05.94	6	0.26	0.16	0.25	0.22	0.28	0.23	0.05	0.02	0.18	0.23	0.19	0.23	0.21	0.03	0.01
02.06.94.	7	0.23	0.19	0.26	0.48	0.14	0.26	0.11	0.05	0.18	0.21	0.20	0.17	0.19	0.02	0.01
09.06.94	8	0.21	0.20	0.41	0.31	0.16	0.26	0.09	0.04	0.27	0.18	0.25	0.22	0.23	0.04	0.02
16.06.94	9	0.29	0.29	0.48	0.35	0.16	0.31	0.08	0.04	0.33	0.15	0.33	0.26	0.27	0.09	0.04
23.06.94	10	0.35	0.28	0.31	0.32	0.19	0.29	0.03	0.01	0.19	0.25	0.26	0.24	0.24	0.03	0.02
30.06.94	11	0.23	0.26	0.35	0.37	0.20	0.28	0.07	0.03	0.27	0.17	0.27	0.26	0.24	0.05	0.02
07.07.94	12	0.26	0.33	0.32	0.38	0.18	0.29	0.04	0.02	0.27	0.22	0.27	0.17	0.23	0.05	0.02
14.07.94	13	0.29	0.14	0.41	0.38	0.25	0.29	0.05	0.02	0.26	0.21	0.27	0.23	0.24	0.03	0.01
21.07.94	14	0.36	0.16	0.48	0.24	0.26	0.30	0.09	0.04	0.25	0.18	0.26	0.24	0.23	0.04	0.02
28.07.94	15	0.28	0.16	0.31	0.27	0.23	0.25	0.02	0.01	0.30	0.25	0.25	0.19	0.25	0.05	0.02
04.08.94	16	0.14	0.19		0.26	0.14	0.18	0.01	0.01	0.31	0.17	0.30	0.15	0.23	0.08	0.04
11.08.94	17	0.16	0.20		0.25	0.16	0.19	0.02	0.01	0.27	0.22	0.33	0.16	0.25	0.07	0.04
18.08.94	18	0.16	0.18		0.30	0.16	0.20	0.06	0.03	0.29	0.21	0.29	0.15	0.24	0.07	0.03
25.08.94	19	0.19	0.25		0.22	0.19	0.21	0.11	0.05	0.29	0.18	0.31	0.16	0.24	0.08	0.04
01.09.94	20	0.20	0.26		0.35	0.20	0.25	0.11	0.05	0.27	0.17	0.25	0.19	0.22	0.05	0.02
08.09.94	21	0.18	0.23		0.33	0.18	0.23	0.08	0.04	0.27	0.23	0.27	0.20	0.24	0.03	0.02
15.09.94	22	0.25	0.21		0.34	0.25	0.26	0.06	0.03	0.27	0.24	0.27	0.25	0.26	0.02	0.01
22.09.94	23	0.26	0.18		0.27	0.26	0.24	0.05	0.03	0.27	0.19	0.26	0.36	0.27	0.07	0.03
29.09.94	24	0.23	0.15		0.29	0.23	0.23	0.07	0.03	0.26	0.15	0.25	0.45	0.28	0.13	0.06
06.10.94.	25	0.21	0.30		0.29	0.21	0.25	0.05	0.02	0.25	0.16	0.30	0.34	0.26	0.08	0.04
13.10.94	26	0.18			0.34	0.18	0.23	0.08	0.06				0.30	0.30		

Appendix Table 4.31:

Eperiment 4: Sheep 23, 25, 27 and 31 infected with *F. gigantica* (Kenyan strain) and sheep 21 and 31 as uninfected controls.

	Glucose (mmol/ml)						β-Hydroxyl-Butyrate (mmol/ml)					
WPI	SH. 23	SH. 25	SH. 27	SH. 29	SH. 21	SH. 31	SH. 23	SH. 25	SH. 27	SH. 29	SH. 21	SH. 31
-2	3.10	3.40	3.54	2.90	3.50	3.50	0.35	0.48	0.37	0.49	0.55	0.33
-1	2.90	3.48	3.21	2.80	3.00	2.80	0.36	0.48	0.70	0.29	0.33	0.47
0	2.40	3.29	2.87	1.80	2.20	2.20	0.36	0.45	0.61	0.43	0.47	0.40
1	2.10	1.63	2.19	1.60	3.48	2.70	0.28	0.53	0.59	0.47	0.46	0.48
2	2.00	2.57	2.47	1.90	3.29	2.50	0.37	0.51	0.35	0.39	0.39	0.39
3	2.30	1.73	2.49	2.00	2.00	2.10	0.41	0.33	0.45	0.47	0.44	0.47
4	1.90	1.41	2.60	1.70	2.10	2.60	0.30	0.46	0.47	0.38	0.42	0.38
5	2.30	1.82	1.80	2.30	2.20	2.30	0.31	0.41	0.48	0.37	0.40	0.44
6	2.10	1.83	2.10	2.10	2.00	2.10	0.43	0.52	0.46	0.39	0.33	0.47
7	2.30	1.63	1.90	1.80	2.00	2.00	0.59	0.54	0.75	0.48	0.47	0.46
8	2.70	1.70	1.70	1.80	2.00	2.30	0.52	0.38	0.45	0.48	0.51	0.39
9	2.90	2.40	2.30	2.20	2.40	2.70	0.44	0.39	0.56	0.45	0.44	0.44
10	1.90	1.63	1.90	1.70	2.50	2.00	0.54	0.48	0.73	0.53	0.35	0.42
11	2.70	2.22	1.10	2.00	2.40	2.50	0.52	0.41	0.50	0.51	0.36	0.40
12	2.50	1.70	2.00	1.80	2.30	2.00	0.58	0.38	0.31	0.33	0.36	0.54
13	2.10	1.33	2.10	1.30	2.50	2.00	0.43	0.41	0.59	0.46	0.28	0.44
14	2.60	1.35	1.30	1.30	2.10	2.10	0.51	0.36	0.53	0.41	0.37	0.46
15	2.30	2.02	2.10	1.90	2.60	2.30	0.39	0.39	0.46	0.52	0.41	0.44
16		2.12	2.00	1.70	2.30	2.50		0.41	0.25	0.54	0.30	0.35
17			1.90	1.20	2.39	2.10			0.44	0.37	0.31	0.36
18			1.96	1.80	2.52	2.60			0.39	0.41	0.31	0.36
19			2.24	1.40	2.59	2.30			0.68	0.39	0.46	0.28
20			1.94	1.80	2.50	1.70			0.46	0.33	0.31	0.37
21			2.11	2.20	2.10	2.00			0.29	0.26	0.35	0.39
22			2.47	1.90	2.60	1.50			0.31	0.35	0.41	0.40

Appendix Table: 4

Experiment 3: Calves 14c, 34c and 45c with single F. hepatica (peruvian strain) infection Calves 15c and 23c with challenge infection and Calf 26c uninfected control.

Appendix Table 4.32:

	Pact Cell Volume (PCV) (%)						Egg per gram (EPG) counts					
WPI	14c	15c	23c	26c	34c	45c	14C	15C	23C	26C	34C	45C
-27	34	32	29	40			0	0	0	0		
-26	33	32	30	38			0	0	0	0		
-25	33	31	30	35			0	0	0	0		
-24	33	30	29	39			0	0	0	0		
-23	36	33	30	32			0	0	0	0		
-22	33	30	30	31			0	0	0	0		
-21	33	31	26	30			0	0	0	0		
-20	35	31	29	30			0	0	0	0		
-19	31	30	29	28			0	0	0	0		
-18	31	30	30	28			0	0	0	0		
-17	32	28	27	28			0	0	0	0		
-16	31	29	26	26			1	0	5	0		
-15	34	28	29	28			3	0	19	0		
-14	33	30	28	27			10	1	50	0		
-13	31	30	26	27			15	3	36	0		
-12	32	30	25	27			11.5	4	45	0		
-11	27	29	24	27			21	3	38	0		
-10	31	30	24	29			77	96	31	0		
-9	29	28	26	28			107	94	54	0		
-8	32	29	26	29			60	54	64	0		
-7	31	28	25	28			56	40	59	0		
-6	27	27	23	28			36	78	41	0		
-5	28	27	25	30			54	39	36	0		
-4	29	28	27	26			32	41	41	0		
-3	27	29	26	28			19	22	34	0		
-2	25	28	26	30			16	10	49	0		
-1	28	28	29	28			21	6	30	0		
0	27	26		29	29	33	18	4	26	0	0	0
1	27	26		31	29	33	15	5	31	0	0	0
2	28	28		28	26	33	18	3	12	0	0	0
3	30	28		31	27	29	13	5	18	0	0	0
6	30	29		28	28	36	12	1		0	0	0
5	30	29		30	27	32	11	2		0	0	0
4	30	31		30	28	30	13	4		0	0	0
7	29	30			31	28	10	2		0	0	0
8	30	30			26	32	15	6		0	4	3
9	28	30			30	31	9	1			6	11
10	29	30					7	0			9	22
11	29	30					8	0				26
12	29	30					4	0				

Appendix Table 4.33:

	Haemoglobin (g/dl.)						RBC counts (x10^{12}/l)					
WPI	14C	15C	23C	26C	34C	45C	14c	15c	23c	26c	34c	45c
-27	13.9	13.0	12.1	16.0			11.055	9.244	7.596	9.602		
-26	13.3	12.0	12.1	14.6			10.056	8.434	8.217	8.575		
-25	14.0	12.4	12.7	13.6			10.210	8.282	9.156	8.808		
-24	14.0	14.2	12.6	13.7			9.510	8.215	8.431	8.561		
-23	14.6	12.0	12.9	14.6			9.334	7.801	7.610	8.123		
-22	12.9	12.6	11.7	12.0			8.892	8.086	7.944	7.767		
-21	14.7	14.6	11.5	11.5			9.383	8.519	7.616	7.622		
-20	13.0	11.5	11.4	13.2			8.254	7.649	7.663	7.549		
-19	13.0	12.5	12.3	11.0			10.117	7.592	7.754	7.840		
-18	13.2	11.3	11.7	12.0			9.745	7.373	7.981	7.030		
-17	12.2	11.3	12.5	12.6			9.843	7.605	7.888	6.743		
-16	15.0	12.5	12.1	10.1			10.832	7.428	6.793	7.119		
-15	13.0	12.0	11.0	12.3			9.718	8.231	7.277	7.051		
-14	12.9	11.6	11.1	10.8			9.324	7.793	9.606	6.792		
-13	13.6	13.4	8.5	11.9			8.815	7.603	6.805	6.682		
-12	12.3	10.9	10.6	10.7			8.758	8.040	6.947	6.992		
-11	12.2	12.1	11.0	12.2			9.100	7.758	6.518	6.891		
-10	11.4	12.1	11.5	11.2			8.580	7.300	5.935	6.907		
-9	12.7	11.6	9.2	10.6			11.029	7.387	6.540	6.684		
-8	12.5	12.7	9.4	11.8			9.887	7.605	7.268	7.818		
-7	10.8	11.7	9.8	11.0			8.416	7.068	7.417	6.571		
-6	10.4	9.8	9.7	11.0			8.033	7.227	7.428	6.775		
-5	10.9	10.5	9.5	11.9			8.400	6.916	6.623	7.042		
-4	11.3	12.2	9.5	12.1			9.933	8.072	7.035	6.448		
-3	11.4	10.4	10.3	11.0			7.625	6.733	7.638	7.468		
-2	11.2	10.9	10.2	10.5			8.116	6.776	7.125	7.196		
-1	12.0	10.7	10.2	10.8			8.498	6.296	5.934	8.278		
0	11.4	10.5	10.6	10.9	11.2	12.0	7.969	6.537	7.409	8.296	8.262	10.925
1	11.0	10.4	10.4	11.9	10.8	12.0	8.045	9.333	7.382	8.081	8.283	10.936
2	11.1	10.2	9.7	10.7	10.0	12.8	8.951	7.780	6.817	7.817	8.603	14.091
3	11.5	11.2		11.5	10.0	11.3	8.986	8.506		9.071	8.311	10.765
6	11.7	11.2		11.0	10.6	14.6	9.054	7.798		7.719	10.406	15.940
5	11.9	11.7		11.3	9.8	11.7	8.773	7.972		7.809	7.991	13.170
4	11.9	11.0		11.2	10.0	11.9	8.361	8.301		8.069	10.676	12.947
7	11.4	11.9		12.1	10.5	12.2	9.058	7.077		8.358	8.708	11.737
8	11.2	11.4		10.8	9.8	12.3	8.364	7.945		7.296	8.226	12.224
9	11.2	11.3			10.8	11.9	8.494	7.225			8.710	11.339
10	11.1	11.3			11.2	12.5	8.270	7.670			8.740	12.052
11	11.6	12.2				12.3	8.590	8.066				11.157
12	11.1	11.4					8.218	7.768				

Appendix Table 4.34:

	MCH (pg)						MCV (fl)						MCHC (g/dl)					
WPI	14c	15c	23c	26c	34c	45c	14c	15c	23c	26c	34c	45c	14c	15c	23c	26c	34c	45c
-27	12.57	14.06	15.93	16.66			30.76	34.62	43.44	41.66			40.88	40.63	36.67	40.00		
-26	13.23	14.23	14.73	17.03			32.82	37.94	45.03	44.31			40.30	37.50	32.70	38.42		
-25	13.71	14.97	13.87	15.44			32.32	37.43	29.49	39.74			42.42	40.00	47.04	38.86		
-24	14.72	17.29	14.94	16			34.70	36.52	34.40	45.56			42.42	47.33	43.45	35.13		
-23	15.64	15.38	16.95	17.97			38.57	42.30	39.42	39.39			40.56	36.36	43.00	45.63		
-22	14.51	15.58	14.73	15.45			37.11	37.10	37.76	39.91			39.09	42.00	39.00	38.71		
-21	15.67	17.14	15.1	15.09			35.17	36.39	38.08	39.36			44.55	47.10	39.66	38.33		
-20	15.75	15.03	14.88	17.49			42.40	40.53	39.15	39.74			37.14	37.10	38.00	44.00		
-19	12.85	16.46	15.86	14.03			30.64	39.52	38.69	35.71			41.94	41.67	41.00	39.29		
-18	13.55	15.33	14.66	17.07			31.81	40.69	32.58	39.83			42.58	37.67	45.00	42.86		
-17	12.39	14.86	15.85	18.69			32.51	36.82	36.76	41.52			38.13	40.36	43.10	45.00		
-16	13.85	16.83	17.81	14.19			28.62	39.04	42.69	36.52			48.39	43.10	41.72	38.85		
-15	13.38	14.58	15.12	17.44			34.99	34.02	41.23	39.71			38.24	42.86	36.67	43.93		
-14	13.84	14.89	11.56	15.9			35.39	38.50	28.11	39.75			39.09	38.67	41.11	40.00		
-13	15.43	17.62	12.49	17.81			35.17	39.46	38.21	40.41			43.87	44.67	32.69	44.07		
-12	14.04	13.56	15.26	15.3			36.54	37.31	41.74	38.62			38.44	36.33	36.55	39.63		
-11	13.41	15.6	16.88	17.7			29.67	37.38	42.96	39.18			45.19	41.72	39.29	45.19		
-10	13.29	16.58	19.38	16.22			36.13	41.10	43.81	41.99			36.77	40.33	44.23	38.62		
-9	11.52	15.7	14.07	15.86			26.29	37.90	38.23	41.89			43.79	41.43	36.80	37.86		
-8	12.64	16.7	12.93	15.09			32.37	38.13	33.02	37.09			39.06	43.79	39.17	40.69		
-7	12.83	16.55	13.21	16.74			36.83	39.62	32.36	42.61			34.84	41.79	40.83	39.29		
-6	12.95	13.56	13.06	16.24			33.61	37.36	35.00	41.33			38.52	36.30	37.31	39.29		
-5	12.98	15.18	14.34	16.9			33.33	39.04	39.26	42.60			38.93	38.89	36.54	39.67		
-4	11.38	15.11	13.5	18.77			29.20	34.69	35.54	40.32			38.97	43.57	38.00	46.54		
-3	14.95	15.45	13.49	14.73			35.41	43.07	30.11	37.49			42.22	35.86	44.78	39.29		
-2	13.8	16.09	14.32	14.59			30.80	41.32	35.09	41.69			44.80	38.93	40.80	35.00		
-1	14.12	16.99	17.19	13.05			32.95	44.47	45.50	33.82			42.86	38.21	37.78	38.57		
0	14.31	16.06	14.31	13.14	13.56	10.98	33.88	39.77	35.09	34.96	31.47	30.21	42.22	40.38	40.77	37.59	0.00	0.00
1	13.67	11.14	14.09	14.73	13.04	10.97	33.56	27.86	35.22	38.36	32.60	26.52	40.74	40.00	40.00	38.39	0.00	0.00
2	12.4	13.11	14.23	13.69	11.62	9.08	31.28	35.99	42.54	35.82	32.55	25.55	39.64	36.43	33.45	38.21	43.08	36.36
3	12.8	13.17		12.68	12.03	10.5	33.39	32.92		34.17	32.49	29.73	38.33	40.00		37.10	40.00	41.38
6	12.92	14.36		14.25	10.19	9.16	33.13	37.19		36.27	26.91	18.82	39.00	38.62		39.29	35.71	35.56
5	13.56	14.68		14.47	12.26	8.88	34.20	36.38		38.42	38.79	21.26	39.67	40.34		37.67	37.04	35.31
4	14.23	13.25		13.88	9.37	9.19	35.88	37.34		37.18	24.35	24.72	39.67	35.48		37.33	37.86	48.67
7	12.59	16.82		14.48	12.06	10.39	32.02	42.39			34.45	26.41	39.31	39.67			31.61	41.79
8	13.39	14.35		14.8	11.91	10.06	35.87	37.76					37.33	38.00			38.46	37.19
9	13.19	15.64			12.4	10.49	32.96	41.52					40.00	37.67			35.00	39.35
10	13.42	14.73			12.81	10.37	35.07	39.11					38.28	37.67				
11	13.5	15.13				11.03	33.76	37.19					40.00	40.67				
12	13.51	14.68					34.35	36.70										

Appendix Table 4.35

	WBC counts (x10^9/l)						Eosinophil counts (x10^9/l)					
WPI	14c	15c	23c	26c	34c	45c	14C	15C	23C	26C	34C	45C
-27	12.162	10.680	4.659	7.830			0.181	0.075	0.031	0.025		
-26	11.670	8.715	5.694	9.724			0.056	0.031	0.006	0.013		
-25	10.773	9.032	8.123	7.679			0.038	0.013	0.000	0.019		
-24	12.259	9.422	8.416	9.010			0.244	0.019	0.038	0.031		
-23	11.643	10.031	6.757	8.470			0.600	0.106	0.575	0.019		
-22	11.450	9.347	8.491	6.500			1.019	0.663	1.855	0.006		
-21	10.671	9.979	8.864	7.677			0.381	0.731	3.206	0.013		
-20	9.979	7.360	10.747	7.442			0.681	0.200	2.819	0.000		
-19	9.758	6.425	10.589	6.576			0.406	0.144	0.019	0.000		
-18	10.884	7.149	7.514	6.746			0.069	0.050	0.000	0.019		
-17	9.479	12.827	7.242	6.980			0.188	0.650	0.063	0.013		
-16	13.916	8.432	8.107	9.504			0.206	0.263	2.538	0.019		
-15	12.157	8.400	7.241	7.591			0.294	0.363	1.975	0.006		
-14	11.482	7.519	8.914	7.244			0.338	0.063	1.750	0.038		
-13	9.007	5.393	8.523	6.008			0.288	0.056	0.969	0.038		
-12	11.255	9.480	6.835	5.020			0.219	0.381	1.550	0.038		
-11	10.676	9.517	8.481	5.776			0.150	0.144	1.313	0.056		
-10	8.837	7.487	8.217	6.842			0.250	0.100	1.381	0.019		
-9	9.239	8.398	8.350	6.058			0.350	0.169	0.925	0.000		
-8	9.827	7.120	8.305	5.833			0.631	0.081	0.994	0.256		
-7	10.080	7.534	7.094	5.343			0.781	0.181	0.925	0.625		
-6	13.117	9.959	7.840	6.404			0.419	0.200	0.813	0.544		
-5	9.757	6.972	7.365	6.127			0.656	0.119	0.819	0.388		
-4	11.424	7.554	6.926	6.536			1.325	0.275	0.956	0.338		
-3	8.007	5.964	7.466	5.955			0.406	0.056	1.394	0.606		
-2	10.576	7.511	7.940	6.363			0.538	0.519	0.931	0.400		
-1	10.552	5.687	7.584	7.001			0.819	0.856	0.331	0.300		
0	9.178	8.287	7.319	6.319	7.302	11.192	0.700	0.900	0.925	0.263	0.000	0.294
1	11.895	8.192	7.505	6.518	9.773	8.244	1.569	0.425	0.606	0.213	0.138	0.088
2	10.526	7.418	7.773	5.588	10.081	11.065	1.363	0.794	0.406	0.275	0.294	0.188
3	9.863	8.588	6.058	6.359	9.279	10.328	0.606	0.456	0.331	0.225	0.756	0.163
6	10.924	9.687		5.470	5.725	14.845	1.006	1.681		0.119	0.519	0.144
5	10.162	9.364		6.504	8.165	8.089	1.206	1.163		0.213	1.225	0.000
4	9.118	9.492		5.470	9.456	9.843	0.881	0.700		0.231	1.494	0.431
7	10.979	10.817		6.710	9.213	11.455	1.063	0.981		0.213	1.194	1.600
8	8.035	8.068		5.492	7.093	13.283	0.494	0.819		0.069	0.706	1.000
9	9.880	8.310		5.492	6.914	10.705	0.319	0.456			1.000	1.169
10	10.147	7.940			6.650	12.319	0.219	0.469			0.388	1.369
11	10.107	8.890				8.049	0.475	1.019			0.000	0.250
12	11.466	8.185					0.863	0.144				

Appendix Table 4.36:

	Neutrophils (x10^9/l)						Lymphocytes (x10^9/l)					
WPI	14c	15c	23c	26c	34c	45c	14c	15c	23c	26c	34c	45c
-27	3.892	4.165	1.305	1.488			7.054	5.233	3.168	5.481		
-26	2.918	1.394	1.537	1.945			6.185	3.050	3.929	6.710		
-25	3.232	5.058	1.950	2.457			6.679	2.890	5.849	4.684		
-24	3.923	3.863	2.188	3.244			6.988	4.617	5.723	4.505		
-23	4.890	4.915	1.081	2.626			5.822	4.313	4.865	4.997		
-22	3.893	4.954	1.359	1.820			6.298	3.739	6.114	3.705		
-21	2.988	2.794	1.418	2.764			6.829	5.588	5.939	4.222		
-20	2.096	2.502	2.257	2.307			6.786	3.974	5.159	4.465		
-19	1.952	2.634	3.706	1.118			7.026	2.956	3.706	4.800		
-18	2.830	2.073	2.029	0.877			7.292	4.075	5.034	5.397		
-17	1.896	6.285	2.680	2.303			6.635	5.516	4.200	3.350		
-16	4.314	3.710	2.027	4.752			8.210	3.794	4.459	2.946		
-15	5.835	3.108	2.824	3.568			5.106	3.948	1.738	3.264		
-14	3.330	3.384	2.229	3.115			7.004	3.684	4.635	3.260		
-13	2.162	1.402	2.216	2.944			5.044	3.020	5.625	2.644		
-12	6.640	3.508	1.025	1.255			3.151	4.076	3.964	3.263		
-11	4.057	3.236	2.544	2.368			5.552	4.663	4.241	3.177		
-10	1.944	2.995	1.561	1.847			5.125	3.369	5.752	4.310		
-9	1.940	2.771	0.919	1.393			5.728	4.535	5.929	4.180		
-8	2.948	2.777	2.575	1.458			5.896	4.058	4.402	2.917		
-7	3.226	3.616	0.426	0.962			4.738	3.541	4.611	3.313		
-6	7.870	6.175	1.803	1.153			4.197	2.589	4.390	4.099		
-5	2.049	1.952	1.694	1.287			6.147	4.880	4.272	3.983		
-4	3.998	2.795	1.732	1.830			5.027	3.928	4.086	3.529		
-3	1.601	2.087	1.419	1.251			5.205	3.221	4.330	3.216		
-2	3.384	2.028	1.429	1.909			4.971	3.605	5.637	3.182		
-1	3.060	0.626	1.896	1.610			6.331	4.152	4.550	4.271		
0	1.285	2.735	3.147	1.580	2.702	2.798	6.241	3.978	3.440	3.539	3.870	7.499
1	3.569	2.376	1.726	2.086	3.714	1.401	6.780	4.096	4.953	3.455	5.668	5.771
2	2.105	1.706	3.031	1.397	3.528	2.988	6.631	3.709	4.120	3.129	5.444	7.635
3	2.367	2.491	0.969	1.081	2.320	2.169	6.805	4.122	4.241	4.197	5.011	6.507
6	1.748	2.712		1.094	0.859	5.790	6.554	4.747		3.610	3.550	8.313
5	2.236	2.903		1.821	1.551	0.485	6.504	4.495		3.707	4.572	6.390
4	1.368	3.607		1.313	1.986	1.870	6.747	4.366		3.446	5.768	7.087
7	1.757	3.678		2.147	2.211	1.146	7.576	4.651		4.227	5.344	8.019
8	1.446	2.662		1.757	1.844	3.321	4.982	4.195		3.460	4.469	8.900
9	2.766	2.909		1.812	2.143	2.462	6.126	3.906		2.911	3.872	6.744
10	4.059	1.667			1.064	1.109	5.175	4.843			4.722	10.348
11	1.415	2.934				1.449	7.985	4.534				5.312
12	4.930	2.783					5.160	4.420				

Appendix Table 4.37:

	Monocytes(x10^9/l)						Eosinophils (x10^9/l)						Basophils (x10^9/l)					
WPI	14c	15c	23c	26c	34c	45c	14c	15c	23c	26c	34c	45c	14c	15c	23c	26c	34c	45c
-27	1.095	1.282	0.186	0.783			0.122	0.000	0.000	0.000			0.000	0.000	0.000	0.078		
-26	0.000	0.174	0.228	0.972			2.567	4.009	0.000	0.000			0.000	0.087	0.000	0.000		
-25	0.646	0.903	0.244	0.538			0.108	0.181	0.081	0.000			0.000	0.000	0.000	0.000		
-24	1.103	0.848	0.505	1.171			0.245	0.094	0.000	0.090			0.000	0.000	0.000	0.000		
-23	0.349	0.802	0.473	0.847			0.582	0.000	0.338	0.000			0.000	0.000	0.000	0.000		
-22	0.687	0.748	0.594	0.975			0.573	0.000	0.425	0.000			0.000	0.000	0.000	0.000		
-21	0.534	1.197	0.443	0.691			0.320	0.299	1.064	0.000			0.000	0.100	0.000	0.000		
-20	0.699	0.662	1.075	0.670			0.299	0.221	2.149	0.000			0.100	0.000	0.000	0.000		
-19	0.585	0.643	0.424	0.592			0.195	0.193	2.753	0.066			0.000	0.000	0.000	0.000		
-18	0.762	0.858	0.451	0.472			0.000	0.071	0.000	0.000			0.000	0.071	0.000	0.000		
-17	0.948	0.770	0.362	1.256			0.000	0.257	0.000	0.070			0.000	0.000	0.000	0.000		
-16	1.392	0.675	0.619	1.711			0.000	0.253	0.973	0.095			0.000	0.000	0.000	0.000		
-15	1.216	0.924	0.290	0.683			0.000	0.420	2.390	0.000			0.000	0.000	0.000	0.000		
-14	0.689	0.451	0.357	0.797			0.459	0.000	1.694	0.072			0.000	0.000	0.000	0.000		
-13	1.531	0.917	0.426	0.360			0.270	0.000	0.256	0.060			0.000	0.054	0.000	0.000		
-12	1.126	1.422	0.684	0.502			0.338	0.379	1.162	0.000			0.000	0.000	0.000	0.000		
-11	1.068	1.618	0.594	0.231			0.000	0.000	1.103	0.000			0.000	0.000	0.000	0.000		
-10	1.502	0.973	0.082	0.547			0.088	0.150	0.822	0.068			0.177	0.000	0.000	0.068		
-9	0.924	0.924	0.501	0.485			0.647	0.168	0.919	0.000			0.000	0.000	0.000	0.000		
-8	0.590	0.285	0.498	0.467			0.393	0.000	0.831	0.875			0.000	0.000	0.000	0.117		
-7	0.504	0.377	0.922	0.481			0.605	0.000	0.993	0.588			0.000	0.000	0.142	0.000		
-6	0.656	0.996	1.019	0.576			0.394	0.199	0.549	0.576			0.000	0.000	0.078	0.000		
-5	1.171	0.139	0.368	0.674			0.390	0.000	1.031	0.184			0.000	0.000	0.000	0.000		
-4	1.257	0.604	0.416	1.046			1.142	0.227	0.693	0.131			0.000	0.000	0.000	0.000		
-3	0.881	0.596	0.373	0.953			0.320	0.060	1.344	0.536			0.000	0.000	0.000	0.000		
-2	1.692	1.427	0.159	0.636			0.529	0.451	0.715	0.636			0.000	0.000	0.000	0.000		
-1	0.317	0.284	0.379	0.840			0.844	0.626	0.758	0.280			0.000	0.000	0.000	0.000		
0	1.101	1.077	0.512	0.758	0.730	0.783	0.551	0.497	0.220	0.379	0.000	0.112	0.000	0.000	0.000	0.063	0.000	0.000
1	0.714	1.311	0.375	0.521	0.391	0.907	0.833	0.410	0.450	0.391	0.000	0.165	0.000	0.000	0.000	0.065	0.000	0.000
2	1.263	1.558	0.466	0.894	0.907	0.443	0.526	0.445	0.155	0.168	0.101	0.000	0.000	0.000	0.000	0.000	0.101	0.000
3	0.592	1.546	0.545	1.017	1.021	1.549	0.099	0.429	0.303	0.064	0.835	0.103	0.000	0.000	0.000	0.000	0.093	0.000
6	1.202	0.969		0.547	0.286	0.742	1.420	1.259		0.219	0.973	0.000	0.000	0.000		0.000	0.057	0.000
5	0.406	1.030		0.911	1.143	1.132	0.915	0.843		0.065	0.817	0.081	0.102	0.094		0.000	0.082	0.000
4	0.547	1.044		0.492	0.662	0.591	0.456	0.475		0.219	1.040	0.295	0.000	0.000		0.000	0.000	0.000
7	0.439	1.298		0.268	1.013	0.916	1.208	1.190		0.067	0.553	1.260	0.000	0.000		0.000	0.092	0.115
8	0.804	0.807		0.220	0.355	0.266	0.804	0.403		0.055	0.426	0.664	0.000	0.000		0.000	0.000	0.133
9	0.593	0.582		0.659	0.346	0.856	0.395	0.914		0.000	0.553	0.642	0.000	0.000		0.110	0.000	0.000
10	0.609	1.032			0.532	0.493	0.304	0.397			0.333	0.370	0.000	0.000			0.000	0.000
11	0.505	1.156				0.885	0.202	0.267				0.402	0.000	0.000				
12	0.344	0.737					1.032	0.246					0.000	0.000				

Appendix Table 4.38:

	Albumin (g/l)						Tota Protein (g/dl)					
WPI	14C	15C	23C	26C	34c	45c	14C	15C	23C	26C	34c	45c
-27	24.30	34.80	34.00	5.70			5.40	5.00	5.70	5.70		
-26	30.80	34.70	35.10	5.50			5.70	5.60	6.10	5.50		
-25	26.00	42.60	38.60	5.10			5.60	6.80	5.70	5.10		
-24	26.90	46.10	37.00	5.10			5.60	7.60	5.70	5.10		
-23	25.80	46.60	40.90	5.40			6.30	5.60	5.70	5.40		
-22	31.20	50.30	38.20	5.20			6.30	6.60	5.60	5.20		
-21	33.70	48.90	35.90	6.40			7.60	5.20	5.50	6.40		
-20	31.40	41.50	43.20	6.80			8.60	7.20	5.60	6.80		
-19	31.40	40.30	33.50	5.10			5.60	5.80	4.20	5.10		
-18	23.00	52.10	36.70	5.30			7.00	5.80	5.70	5.30		
-17	28.00	34.80	41.50	5.90			6.60	6.10	5.70	5.90		
-16	34.10	36.80	41.30	8.00			7.80	6.20	6.10	8.00		
-15	29.00	45.80	37.70	5.80			6.10	6.40	6.00	5.80		
-14	28.60	38.30	36.70	5.60			10.10	5.70	6.00	5.60		
-13	28.30	39.40	38.90	5.80			7.40	5.70	6.10	5.80		
-12	26.30	42.50	38.50	5.30			8.10	6.70	6.00	5.30		
-11	37.10	37.20	38.90	6.10			6.20	5.80	6.50	6.10		
-10	30.20	39.50	32.50	5.90			6.60	7.00	6.80	5.90		
-9	33.90	39.10	35.40	5.60			8.00	5.60	6.30	5.60		
-8	22.80	41.20	35.90	5.90			6.60	5.40	6.70	5.90		
-7	37.80	44.40	47.70	4.90			8.50	5.90	6.20	4.90		
-6	36.60	42.40	35.10	6.40			6.10	7.20	6.90	6.40		
-5	31.10	35.20	32.80	7.50			5.70	7.70	8.40	7.50		
-4	32.50	38.60	30.40	5.50			5.60	5.30	10.40	5.50		
-3	36.40	40.50	29.70	5.40			5.80	5.70	12.50	5.40		
-2	32.30	33.10	27.50	6.00			7.10	5.80	7.80	6.00		
-1	32.70	40.50	36.50	5.10			6.10	6.30	9.90	5.10		
0	35.00	39.80	36.00	5.30	25.50	38.40	6.10	5.70	8.00	5.30	6.70	10.20
1	26.70	35.20	34.60	5.70	34.40	34.30	5.70	9.50	9.40	5.70	6.70	6.20
2	29.10	34.90	36.80	5.40	25.60	31.00	6.10	5.10	8.70	5.40	9.70	6.60
3	27.30	35.60	34.60	5.80	23.20	32.70	6.30	10.30	8.30	5.80	4.60	7.10
6	31.70	39.40	27.30	5.40	29.00	27.40	6.20	10.50	7.20	5.40	6.60	7.40
5	26.20	36.80	36.30	5.60	24.60	37.20	7.70	6.10	7.70	5.60	7.60	9.20
4	24.40	39.80	35.40	6.10	24.30	28.40	8.20	9.20	8.70	6.10	6.70	9.60
7	27.80	42.90	39.60	5.60	29.20	30.20	8.30	10.60	7.00	5.60	7.60	8.70
8	37.60	36.90	34.60		29.20	30.70	6.50	9.30	5.80		8.80	7.70
9	27.60	37.70	37.00		28.90	34.10	9.40	7.70	10.70		6.50	9.30
10	25.10	39.70	32.60		36.10	34.50	7.80	8.10	10.50		8.20	10.20
11	37.60	41.90	36.20		33.10	41.30	6.10	8.60	10.60		6.40	11.20
12	27.30	40.00	37.80		29.70	32.40	9.70	8.80	12.50		5.60	6.00
38		37.40	29.50					7.40	10.10			

Appendix Table 4.39:

	Glutamate dehydrogenase (IU)						Gamma Glutamyltransferase (IU)					
WPI	14C	15C	23C	26C	34c	45c	14C	15C	23C	26C	34c	45c
-27	34.9	19	3.3	24.7			24.3	9.3	17.8	8.5		
-26	15	17.3	5.7	30			30.8	11.2	14.7	8.5		
-25	23.2	12	17.9	19.2			26	13.5	15.1	6.9		
-24	23.4	26	7	9.9			26.9	10.8	11.6	10		
-23	45.3	50	9.8	17.2			25.8	14.7	11.6	10.8		
-22	43.9	45.3	36.4	13.4			31.2	14.3	11.2	10		
-21	86	18.1	24.4	20			33.7	13.9	12	12		
-20	72.3	24	17.5	8			31.4	12.7	12	10.8		
-19	56.5	52	14.2	19			31.4	13.5	9.8	12		
-18	167.8	28.6	23.8	9			23	13.5	15.8	11.6		
-17	108.5	58	29.1	11			28	19	12.4	12.7		
-16	164.7	110.5	15	15.8			34.1	35.5	12.7	8.9		
-15	99.5	65	31.3	21.9			29	33.6	9.3	13.5		
-14	108	65	26.4	24			28.6	65.6	11.2	13.5		
-13	43.1	44	20.1	19			28.3	21.2	11.2	14.3		
-12	25.2	34.7	35.9	20.3			26.3	20.5	11.2	13.1		
-11	25.2	32.9	43.9	21.9			37.1	22.8	11.6	15		
-10	23.4	20.5	34.1	20.3			30.2	13.9	10.8	15.1		
-9	28.2	21.7	58.7	16.8			33.9	13.9	13.8	12.7		
-8	41.4	17.3	51.7	17.4			22.8	14.7	14.7	13.9		
-7	28.2	9.9	110.2	9.9			37.8	13.5	15.1	12		
-6	64.8	39	106.8	14.1			36.6	23.9	25.5	15.4		
-5	42.6	23.4	101.7	30			31.1	15.1	24.7	14.3		
-4	39.6	12.6	111.3	9			32.5	13.1	34	14.3		
-3	52.4	42.1	92.8	26.7			36.4	17	29.3	14.7		
-2	81.6	24.4	121.4	8.9			32.3	21.2	26.6	12.7		
-1	35.3	8.7	108.2	25.5			32.7	14.3	41.7	13.5		
0	46.5	6.6	99.9	15.2	8.3	12.2	35	16.6	39.4	13.1	10.4	12
1	20.7	8.7	42.9	21.2	3.2	13.2	26.7	14.7	31.7	12.4	11.2	12.4
2	21.1	14.4	42.9	17.3	6.3	21	29.1	14.3	25.5	13.1	12.7	12
3	19.7	13	64	8.9	19.1	69	27.3	13.9	24.3	12.4	11.6	12.7
6	11	29.7	28.2	19	34	67.3	31.7	23.2	21.2	11.6	12	17
5	24.2	11.8	22.9	11.3	14.6	116	26.2	16.6	20.1	12.7	12.4	22
4	18.5	9.3	35.7	14.4	13	38.2	24.4	18.1	16.0	12.4	14.3	18.9
7	9.3	4.3	15.8	17.1	42	142.4	27.8	12	16.2	12.7	13.1	30.9
8	6.1	7.1	16.5		46.7	101.8	37.6	15.4	15.8		19.3	43.2
9	10	13.2	12.8		52.8	87.7	27.6	13.5	12.4		19.7	40.5
10	18.3	12.1	39.4		57.8	66.2	25.1	13.5	15.1		21.6	36.7
11	15.2	9.9	10.4		68.3	130	37.6	14.7	12.4		29	70
12	21.1	9.3	9.9		127.8	45.5	27.3	14.7	12		19.7	49.4
13		6.7	7.7					15.4	15.1			

Appendix Table 4.40:

	Glucose (mmol/ml)						β-Hydroxyl-Butyrate (mmol/ml)					
W P I	14C	15C	23C	26C	34C	45C	14C	15C	23C	26C	34C	45C
-27	1.10	3.20	2.93	2.20			0.15	0.58	0.39	0.46		
-26	1.00	2.60	1.31	3.10			0.26	0.51	0.21	0.59		
-25	2.20	1.30	1.72	2.58			0.34	0.20	0.47	0.59		
-24	2.70	3.10	1.80	2.63			0.34	0.42	0.28	0.66		
-23	2.30	3.00	2.64	1.69			0.35	0.38	0.34	0.20		
-22	2.90	3.70	2.15	1.41			0.36	0.43	0.40	0.24		
-21	1.90	2.40	3.19	1.66			0.38	0.39	0.70	0.34		
-20	2.20	4.00	1.43	0.83			0.31	0.45	0.52	0.23		
-19	2.50	1.70	2.24	0.21			0.30	0.16	0.36	0.21		
-18	2.50	1.60	2.03	1.35			0.37	0.17	0.31	0.13		
-17	1.70	1.90	2.16	1.97			0.28	0.36	0.18	0.23		
-16	1.40	1.10	1.38	2.16			0.45	0.17	0.18	0.27		
-15	3.90	1.00	0.48	0.78			0.24	0.07	0.09	0.18		
-14	2.20	2.10	2.11	2.21			0.42	0.26	0.21	0.26		
-13	2.50	2.10	1.17	2.09			0.36	0.19	0.14	0.23		
-12	2.80	2.70	1.84	1.84			0.29	0.26	0.26	0.21		
-11	1.50	1.30	2.33	1.45			0.32	0.28	0.27	0.49		
-10	2.60	2.20	0.81	0.83			0.34	0.24	0.21	0.18		
-9	2.70	1.50	1.68	1.53			0.37	0.15	0.23	0.20		
-8	2.30	1.80	2.78	2.41			0.29	0.18	0.25	0.27		
-7	0.80	1.50	1.70	1.56			0.23	0.37	0.16	0.12		
-6	0.50	1.00	1.05	2.33			0.25	0.21	0.24	0.14		
-5	2.40	2.30	1.18	1.89			0.25	0.26	0.33	0.19		
-4	2.10	1.30	2.55	1.91			0.20	0.09	0.37	0.19		
-3	2.40	1.40	2.15	1.21			0.27	0.17	0.19	0.18		
-2	1.80	2.30	1.90	1.94			0.28	0.29	0.25	0.15		
-1	2.10	2.60	2.25	2.37			0.15	0.23	0.30	0.18		
0	2.10	1.90	2.87	2.64	2.80	2.15	0.20	0.18	0.24	0.19	0.30	0.36
1	2.40	1.90	2.66	1.64	1.60	0.94	0.20	0.20	0.24	0.23	0.31	0.22
2	1.20	1.80	3.05	2.84	2.70	1.96	0.16	0.19	0.28	0.25	0.37	0.26
3	1.80	3.00	1.92	3.41	3.00	2.44	0.21	0.26	0.18	0.26	0.42	0.35
6	1.70	2.20	2.21	3.14	3.50	2.49	0.13	0.20	0.17	0.17	0.29	0.43
5	1.00	2.40	2.65	3.00	0.20	1.90	0.05	0.27	0.20	0.28	0.26	0.24
4	0.20	1.20	2.41	2.33	2.70	2.90	0.08	0.21	0.20	0.25	0.12	0.34
7	1.10	1.80	1.69	2.70	1.60	2.90	0.10	0.18	0.15	0.19	0.16	0.35
8	1.60	3.10	2.35		2.20	2.94	0.13	0.32	0.20	0.24	0.14	0.47
9	1.40	3.70	3.86		2.40	3.22	0.10	0.35	0.28		0.25	0.41
10	0.70	2.40	2.29		3.60	2.67	0.05	0.16	0.18		0.28	0.34
11	1.00	2.00	3.16			3.29	0.09	0.14	0.23		0.21	0.38
12	1.10	1.70	2.45				0.10	0.16	0.19			

Appendix Table 4.41

Experiment 5: Calves 22, 23 and 24 infected with 400 F. gigantica metacercariae (kenyan strain) and uninfected control Calf 26.

	Packed Cell Volume(%)				Haemoglobin in g/dl				Red Blood Cell x1012/l			
WPI	Calf 22	Calf 23	Calf 24	Calf 26	Calf 22	Calf 23	Calf 24	Calf 26	Calf 22	Calf 23	Calf 24	Calf 26
-2	27	34	29	29	13.5	14.1	13.2	12.4	5.120	7.420	6.450	7.040
-1	33	33	33	34	13.4	14.2	13.4	12.9	5.800	9.930	7.420	17.570
0	31	38	34	34	14.2	16.0	17.3	16.6	8.040	10.090	6.900	11.110
1	30	29	33	28	14.7	15.4	14.2	15.4	5.300	7.790	5.400	9.060
2	32	38	34	35	14.2	18.5	16.5	18.6	7.980	9.710	5.940	7.960
3	35	36	33	35	14.6	14.6	16.5	17.4	7.740	7.910	7.550	7.740
4	33	30	35	32	14.2	16.7	16.0	15.4	7.630	9.070	7.060	9.320
5	34	33	34	34	14.7	15.5	16.6	16.0	9.950	7.980	6.220	8.640
6	32	32	33	33	14.7	16.7	16.0	16.0	14.670	11.830	7.850	7.160
7	35	34	34	32	15.4	14.1	15.4	14.8	8.750	7.680	9.230	8.470
8	30	31	30	32	13.0	13.5	13.5	15.4	8.440	9.800	7.330	7.800
9	32	30	32	31	13.5	13.5	14.1	15.4	8.820	13.550	6.200	8.720
10	32	31	34	30	13.0	14.1	13.4	16.0	9.620	8.260	6.950	7.570
11	33	35	34	35	12.7	13.5	13.0	14.8	10.190	9.380	7.190	9.220
12	31	31	33	31	11.2	11.9	12.1	15.0	7.390	7.730	8.260	8.500
13	26	31	33	33	12.4	12.4	12.4	14.9	10.120	8.650	7.160	7.390
14	30	32	26	30	12.4	13.5	13.2	14.5	7.080	8.480	5.800	7.380
15	29	33	31	31	12.4	14.0	13.5	14.5	8.540	13.960	6.500	7.430
16	29	28	32	34	13.0	14.2	13.7	15.4	6.160	6.110	7.080	6.250
17	34	34	33	35	13.0	14.0	13.7	14.9	7.990	7.390	6.900	7.520
18	31	29	30	33	13.8	14.2	13.5	15.4	8.720	6.170	5.960	10.810
19	33	31	32	34	16.2	14.2	17.0	15.3	9.150	6.360	5.900	7.380
20	31	32	35	35	16.2	18.0	16.0	16.0	5.720	6.150	6.640	6.500
21	33	35	34	36	14.7	17.3	16.7	16.0	6.800	10.840	6.440	7.120
22	30	35	33	31	14.7	16.6	14.7	14.2	10.110	7.630	5.880	5.970
23	34	35	33	39	16.0	16.0	18.0	15.3	13.350	8.050	15.020	12.130
24	28	30	33	33	15.5	15.4	16.6	15.3	6.210	8.460	7.330	7.980
25	35	38	37	36	16.0	18.0	18.0	15.3	7.470	7.730	6.860	6.620
26	38	34	38	36	17.0	17.0	18.0	16.0	6.930	8.320	7.710	8.150
27	35	36	30	37	16.0	16.6	17.3	17.3	6.910	9.300	6.040	7.250
28	35	34	34	35	16.0	16.0	17.3	16.0	6.420	7.300	7.130	9.290
29	35	35	37	31	16.0	17.3	15.4	14.6	6.110	12.340	12.000	7.820
30	33	33	36	36	13.5	14.7	13.5	14.7	7.310	6.750	6.650	7.420
31	36	35	37	35	14.1	16.6	17.4	16.6	9.580	8.720	8.920	7.740
32	31	30	34	33	14.1	16.0	16.0	14.1	7.700	7.310	7.180	8.970
33	32			31	14.6			14.1	7.700	8.000	6.900	

Appendix Table 4.42:

	MCV (x10^6/ml.)				MCH (x10^6/ml.)				MCHC (x10^6/ml.			
WPI	Calf 22	Calf 23	Calf 24	Calf 26	Calf 22	Calf 23	Calf 24	Calf 26	Calf 22	Calf 23	Calf 24	Calf 26
-2	52.7	45.8	45	41.2	26.4	19	20.5	17.6				
-1	56.9	33.2	44.5	19.4	23.1	14.3	18.1	7.3	26.4	19	20.5	17.6
0	38.6	37.7	49.3	30.6	17.7	15.9	25.1	14.9	23.1	14.3	18.1	7.3
1	56.6	37.2	61.1	30.9	27.7	19.8	26.3	17	17.7	15.9	25.1	14.9
2	40.1	39.1	57.2	44	17.8	19.1	27.8	23.4	27.7	19.8	26.3	17
3	45.2	45.5	43.7	45.2	18.9	18.5	21.9	22.5	17.8	19.1	27.8	23.4
4	43.3	33.1	49.6	34.3	18.6	18.4	22.7	16.5	18.9	18.5	21.9	22.5
5	34.2	41.4	54.7	39.4	14.8	19.4	26.7	15	18.6	18.4	22.7	16.5
6	21.8	27	42	46.1	10	14.1	20.4	22.3	14.8	19.4	26.7	15
7	40	44.3	36.8	37.8	17.6	18.4	16.7	17.5	10	14.1	20.4	22.3
8	35.5	31.6	40.9	41	15.4	13.8	18.4	17.3	17.6	18.4	16.7	17.5
9	36.3	22.1	51.6	35.6	15.3	10	22.7	17.7	15.4	13.8	18.4	17.3
10	33.3	37.5	48.9	39.6	13.5	17.1	23	17.7	15.3	10	22.7	17.7
11	32.4	37.3	47.3	38	14.4	14.4	21.4	16.1	13.5	17.1	23	17.7
12	41.9	40.1	40	36.5	15.2	15.4	14.6	14.5	14.4	14.4	21.4	16.1
13	25.7	35.8	46.1	44.7	12.3	14.3	17.3	16.8	15.2	15.4	14.6	14.5
14	42.4	37.7	44.8	40.7	17.5	15.9	22.8	17.6	12.3	14.3	17.3	16.8
15	34	23.6	47.7	41.7	14.5	10	20.8	18.8	17.5	15.9	22.8	17.6
16	47.1	45.8	45.2	54.4	21.1	23.2	19.4	21.1	14.5	10	20.8	18.8
17	42.6	46	47.8	46.5	16.3	18.9	19.9	18.9	21.1	23.2	19.4	21.1
18	35.6	47	50.3	30.5	15.8	23	22.7	13.4	16.3	18.9	19.9	18.9
19	36.1	48.7	54.2	46.1	17.7	22.3	28.8	19	15.8	23	22.7	13.4
20	54.2	52	52.7	53.8	28.3	29.3	24.1	24.6	17.7	22.3	28.8	19
21	48.5	32.3	52.8	50.6	21.6	16	25.9	22.5	28.3	29.3	24.1	24.6
22	29.7	45.9	56.1	51.9	14.5	21.8	25	23.8	21.6	16	25.9	22.5
23	25.5	43.5	22	32.2	12	19.9	12	12.6	14.5	21.8	25	23.8
24	45.1	35.5	45	41.4	25	18.2	22.6	19.2	12	19.9	12	12.6
25	46.9	49.2	53.9	54.4	21.4	23.3	26.2	23.1	25	18.2	22.6	19.2
26	54.8	40.9	49.3	44.2	24.5	20.4	23.3	19.6	21.4	23.3	26.2	23.1
27	50.7	38.7	49.7	51	23.2	17.8	28.6	23.9	24.5	20.4	23.3	19.6
28	54.5	46.6	47.7	37.7	24.9	21.9	24.3	17.2	23.2	17.8	28.6	23.9
29	57.3	28.4	30.8	39.6	26.2	14	12.8	18.7	24.9	21.9	24.3	17.2
30	45.1	48.9	54.1	48.5	18.5	21.8	20.3	19.8	26.2	14	12.8	18.7
31	37.6	40.1	41.5	45.2	14.7	19	19.5	21.4	18.5	21.8	20.3	19.8
32	40.3	41	47.4	36.8	18.3	21.9	22.3	15.7	14.7	19	19.5	21.4
33	39.4			36.6	18			16.6	18.3	21.9	22.3	15.7

Appendix Table 4.43:

	WBC ($x10^9$/l)				Eosinophils ($x10^9$/ml.)				Nuetrophil ($x10^9$/l)			
WPI	Calf 22	Calf 23	Calf 24	Calf 26	Calf 22	Calf 23	Calf 24	Calf 26	Calf 22	Calf 23	Calf 24	Calf 26
-2	9.050	7.400	7.700	7.800	0.181	0.074	0.231	0.078	3.258	2.072	1.386	2.652
-1	9.200	6.850	9.100	5.150	0.184	0.069	0.273	0.103	2.852	1.850	1.638	1.648
0	9.500	7.550	6.850	5.500	0.190	0.076	0.137	0.055	2.375	1.812	1.233	2.090
1	8.050	4.050	5.400	4.950	0.483	0.041	0.054	0.050	2.737	1.215	1.188	1.980
2	9.300	6.450	9.450	6.200	0.837	0.194	1.701	0.062	2.511	1.742	2.174	2.046
3	8.700	8.400	11.700	5.750	0.957	0.672	2.340	0.173	1.740	2.184	2.457	1.840
4	9.100	8.350	7.400	6.500	1.274	1.002	1.184	0.065	2.912	1.754	1.628	2.405
5	2.500	5.250	6.850	6.400	0.175	0.368	0.480	0.064	0.800	1.260	1.713	2.496
6	10.100	12.250	10.450	5.050	0.404	1.960	1.359	0.101	3.333	3.675	2.090	1.566
7	8.950	5.650	12.200	7.100	0.537	0.283	2.074	0.142	2.417	1.074	2.684	2.414
8	11.350	7.200	8.250	5.800	1.022	0.576	0.660	0.116	2.384	1.368	1.403	1.856
9	10.950	7.450	11.550	5.550	1.205	1.118	1.733	0.056	2.519	1.639	1.386	1.721
10	10.050	5.600	11.300	5.600	1.508	0.448	1.130	0.112	1.608	1.232	0.904	1.680
11	12.250	10.900	10.200	5.100	0.858	1.417	1.938	0.051	2.205	1.635	2.754	1.326
12	13.200	10.300	13.800	7.000	1.716	2.060	1.656	0.420	3.036	1.854	2.760	1.820
13	10.450	9.100	9.450	4.050	1.150	1.820	1.512	0.041	2.299	1.638	0.662	1.458
14	10.600	9.950	12.900	7.050	1.060	1.990	3.483	0.141	2.120	1.095	1.032	1.622
15	7.450	8.200	9.750	9.000	0.671	0.738	2.730	0.090	1.937	1.476	1.268	2.700
16	13.600	8.800	11.050	5.350	0.272	0.880	2.652	0.168	2.720	1.144	0.332	0.910
17	13.450	8.250	9.750	5.400	0.135	0.660	1.560	0.054	1.480	2.145	0.683	1.890
18	12.200	5.650	9.900	4.350	0.610	0.735	1.188	0.087	0.488	1.300	0.495	0.609
19	12.150	8.050	6.850	5.350	0.608	1.127	0.274	0.054	3.038	1.852	0.891	0.910
20	10.450	9.250	13.400	5.900	0.627	1.758	1.474	0.118	2.404	2.590	2.948	1.180
21	10.700	10.750	10.000	6.100	0.642	2.795	1.100	0.183	2.247	1.935	1.600	1.586
22	14.150	9.500	8.700	5.050	0.283	1.615	1.305	0.101	3.538	1.140	0.870	1.111
23	10.600	9.650	9.900	6.700	1.166	1.641	1.485	0.067	0.954	1.544	1.683	1.139
24	12.700	11.250	9.150	4.950	0.127	0.225	1.739	0.050	2.413	3.938	0.915	0.941
25	11.850	10.050	10.150	6.550	0.356	0.603	1.320	0.066	2.607	2.513	0.711	1.572
26	11.500	7.700	16.350	5.750	0.920	0.385	2.780	0.230	2.875	1.617	2.780	1.840
27	11.000	6.950	12.650	8.150	0.440	0.556	1.518	0.082	2.860	1.599	0.886	2.119
28	12.200	8.500	11.200	8.750	0.244	0.765	1.120	0.175	3.416	2.210	2.128	2.713
29	12.500	8.850	10.300	8.000	0.875	0.708	1.854	0.080	2.125	2.213	0.515	1.680
30	9.800	8.500	13.550	7.150	0.196	0.765	2.981	0.072	1.862	2.040	2.168	3.075
31	11.900	10.650	12.050	7.750	0.119	0.320	1.446	0.078	2.618	3.515	1.687	1.705
32	14.700	8.450	12.750	8.750	0.588	0.169	1.148	0.088	2.499	1.268	2.933	2.713
33	10.200			8.750	0.510			0.350	2.142			1.838

Appendix Table 4.44:

	Lyphocytes (x10^9/ml.)				Monocytes (x10^9/ml.)				Basophils (x10^9/ml.)			
WPI	Calf 22	Calf 23	Calf 24	Calf 26	Calf 22	Calf 23	Calf 24	Calf 26	Calf 22	Calf 23	Calf 24	Calf 26
-2	4.435	4.514	5.236	4.290	0.996	0.740	0.847	0.702	0.181	0.000	0.000	0.078
-1	5.060	4.179	6.188	2.781	1.104	0.754	0.910	0.618	0.000	0.000	0.091	0.000
0	5.795	5.059	5.001	3.080	1.045	0.604	0.411	0.220	0.095	0.000	0.069	0.055
1	4.025	2.349	3.996	2.525	0.805	0.365	0.162	0.347	0.000	0.081	0.000	0.050
2	5.115	4.257	4.631	3.410	0.744	0.258	0.945	0.682	0.093	0.000	0.000	0.000
3	4.698	4.956	6.318	2.990	1.305	0.588	0.585	0.748	0.000	0.000	0.000	0.000
4	4.368	5.177	4.366	3.705	0.546	0.418	0.222	0.325	0.000	0.000	0.000	0.000
5	1.375	3.308	4.384	3.456	0.150	0.315	0.274	0.384	0.000	0.000	0.000	0.000
6	5.555	6.493	6.479	3.081	0.808	0.123	0.523	0.303	0.000	0.000	0.000	0.000
7	5.549	3.955	7.198	4.189	0.448	0.339	0.244	0.355	0.000	0.000	0.000	0.000
8	6.810	4.752	5.528	3.074	1.135	0.504	0.578	0.754	0.000	0.000	0.083	0.000
9	6.023	4.247	7.392	3.441	1.205	0.447	1.040	0.333	0.000	0.000	0.000	0.000
10	6.030	3.752	8.814	3.136	0.804	0.168	0.339	0.672	0.101	0.000	0.113	0.000
11	7.963	7.303	5.202	3.264	1.348	0.545	0.306	0.459	0.000	0.000	0.000	0.000
12	7.656	5.459	8.556	4.060	0.792	0.927	0.828	0.700	0.000	0.000	0.000	0.000
13	6.897	5.642	6.804	2.187	0.105	0.910	0.473	0.365	0.000	0.000	0.000	0.000
14	6.784	5.572	7.998	4.230	0.636	1.294	0.387	1.058	0.000	0.000	0.000	0.000
15	4.321	4.756	5.265	5.760	0.522	1.230	0.390	0.450	0.000	0.000	0.098	0.000
16	10.064	6.072	7.514	3.638	0.544	0.704	0.442	0.535	0.000	0.000	0.111	0.000
17	9.953	4.703	6.825	3.186	1.883	0.743	0.683	0.270	0.000	0.000	0.000	0.000
18	9.394	2.995	7.326	3.132	1.708	0.622	0.891	0.522	0.000	0.000	0.000	0.000
19	7.776	4.186	5.412	3.799	0.729	0.886	0.274	0.589	0.000	0.000	0.000	0.000
20	6.584	4.070	8.576	3.953	0.836	0.833	0.402	0.649	0.000	0.000	0.000	0.000
21	6.848	5.160	7.000	4.148	0.856	0.860	0.300	0.183	0.107	0.000	0.000	0.000
22	9.198	5.985	6.264	3.485	1.132	0.760	0.261	0.303	0.000	0.000	0.000	0.051
23	8.268	5.790	6.336	4.824	0.212	0.676	0.396	0.670	0.000	0.000	0.000	0.000
24	9.271	6.075	5.948	3.366	0.889	1.013	0.549	0.545	0.000	0.000	0.000	0.000
25	8.295	6.734	7.816	4.585	0.474	0.201	0.203	0.328	0.119	0.000	0.102	0.000
26	6.555	5.005	9.647	3.163	1.150	0.616	0.981	0.518	0.000	0.077	0.164	0.000
27	6.930	4.379	9.994	5.053	0.770	0.417	0.253	0.897	0.000	0.000	0.000	0.000
28	7.442	4.335	6.496	4.725	1.098	1.190	0.560	1.138	0.000	0.000	0.000	0.000
29	9.375	5.222	7.416	4.960	0.125	0.708	0.515	1.280	0.000	0.000	0.000	0.000
30	6.468	5.015	7.046	3.361	1.274	0.680	1.355	0.644	0.000	0.000	0.000	0.000
31	8.211	6.284	8.194	5.038	0.952	0.533	0.723	0.930	0.000	0.000	0.000	0.000
32	10.143	5.915	7.650	4.900	1.470	1.099	1.020	1.050	0.000	0.000	0.000	0.000
33	6.732			5.163	0.816			1.400	0.000			0.000

Appendix Table 4.45:

	Glucose (mmol/ml)				β-Hydroxyl-Butyrate (mmol/ml)			
W P I	14C	15C	23C	26C	22	23	24	26
-2	2.80	3.20	2.93	2.20	0.33	0.26	0.29	0.22
-1	1.60	2.60	2.15	3.10	0.31	0.31	0.27	0.21
0	2.70	1.30	1.94	2.58	0.31	0.26	0.18	0.18
1	3.00	3.10	1.96	2.63	0.25	0.35	0.26	0.25
2	3.50	3.00	2.44	3.70	0.29	0.32	0.23	0.28
3	2.70	3.70	2.49	2.40	0.26	0.24	0.21	0.24
4	1.60	2.40	1.90	2.00	0.31	0.34	0.23	0.34
5	2.20	4.00	2.90	2.80	0.37	0.35	0.27	0.23
6	2.40	3.70	2.90	1.60	0.33	0.29	0.42	0.21
7	3.60	2.40	2.94	2.70	0.29	0.38	0.44	0.28
8	1.70	2.00	3.22	3.00	0.47	0.34	0.50	0.23
9	1.40	1.70	2.67	3.50	0.41	0.38	0.49	0.27
10	3.90	1.00	3.29	2.90	0.35	0.47	0.39	0.30
11	2.20	2.10	2.11	2.90	0.43	0.26	0.35	0.26
12	2.50	2.10	1.17	2.94	0.47	0.41	0.43	0.23
13	2.80	2.70	1.84	3.22	0.41	0.26	0.24	0.21
14	1.50	1.30	2.33	2.67	0.35	0.28	0.34	0.22
15	2.60	2.20	0.81	2.70	0.34	0.24	0.35	0.18
16	2.70	1.50	1.68	3.00	0.37	0.49	0.47	0.20
17	2.30	1.80	2.78	3.50	0.29	0.28	0.41	0.27
18	0.80	1.50	1.70	1.56	0.23	0.37	0.34	0.12
19	0.50	1.00	1.05	2.33	0.25	0.21	0.38	0.14
20	2.40	2.30	1.18	2.35	0.25	0.26	0.33	0.19
21	2.10	1.30	2.55	3.86	0.20	0.09	0.37	0.20
22	2.40	1.40	2.15	2.29	0.27	0.17	0.19	0.20
23	1.80	2.30	1.90	3.16	0.28	0.29	0.25	0.16
24	2.10	2.60	2.25	2.45	0.15	0.23	0.30	0.21
25	2.10	1.90	2.87	2.64	0.20	0.18	0.24	0.13
26	2.40	1.90	2.66	1.64	0.20	0.20	0.24	0.25
27	1.20	1.80	3.05	2.84	0.16	0.19	0.28	0.19
28	1.80	3.00	1.92	3.41	0.21	0.26	0.18	0.26
29	1.70	2.20	2.21	3.14	0.13	0.20	0.17	0.17
30	1.00	2.40	2.65	3.00	0.25	0.27	0.20	0.28
31	0.20	1.20	2.41	2.33	0.19	0.24	0.20	0.25
32	1.10	1.80	1.69	2.70	0.20	0.24	0.15	0.19
33	1.60	3.10	2.35	3.86	0.23	0.28	0.20	0.24

Appendix Table:4

OPTICAL DENSITIES AT 450 NM FOR ELISA TITRATIONS

Titration for total Ig showing the mean OD (450 nm) values obtained in *F. hepatica* and *F. gigantica* infected and uninfected sheep

Appendix Table 4.46:

Antigen (μg/ml)

F. hepatica					*F. gigantica*				
FhESP	B	N	P1	P2	FgESP	B	N	P1	P2
20	0.2105	0.4883	1.634	1.580	20	0.097	0.393	1.151	1.304
10	0.2281	0.4586	1.497	1.457	10	0.101	0.292	1.039	1.182
5	0.2187	0.3023	1.415	1.258	5	0.101	0.220	0.866	0.982
2.5	0.1797	0.25	1.258	1.214	2.5	0.075	0.122	0.742	0.723
1.25	0.1663	0.157	1.0623	0.914	1.25	0.088	0.215	0.547	0.428
0.625	0.188	0.25	0.82	0.541	0.625	0.071	0.124	0.337	0.313
0.3125	0.192	0.09	0.6063	0.340	0.3125	0.060	0.098	0.214	0.119
BBS	0.1408	0.3023	0.3366	0.124	BBS	0.040	0.078	0.072	0.086

Serum titrations

F. hepatica					*F. gigantica*				
Serum	B	N	P1	P2	Serum	B	N	P1	P2
100	0.005	0.647	1.424	1.308	100	0.099	0.569	0.984	0.987
200	0.011	0.474	1.257	1.261	200	0.101	0.451	0.884	0.800
400	0.009	0.274	1.003	1.058	400	0.101	0.324	0.766	0.714
800	0.015	0.175	0.715	0.826	800	0.097	0.253	0.627	0.600
1600	0.025	0.104	0.454	0.518	1600	0.103	0.176	0.402	0.458
3200	0.014	0.066	0.285	0.324	3200	0.096	0.155	0.316	0.231

Conjugate titration

F. hepatica					*F. gigantica*				
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.104	0.154	0.852	1.304	1000	0.084	0.183	0.682	0.994
2000	0.057	0.159	0.582	1.182	2000	0.076	0.165	0.471	0.765
4000	0.011	0.066	0.295	0.982	4000	0.046	0.154	0.274	0.424
8000	0.004	0.027	0.139	0.723	8000	0.028	0.089	0.151	0.180
16000	0.005	0.017	0.060	0.428	16000	0.013	0.042	0.085	0.022
32000	0.002	0.007	0.026	0.313	32000	0.006	0.023	0.050	-0.006

Appendix Table 4.47

Titrations for IgG_1 showing the mean OD (450 nm) values obtained in *F. hepatica* and *F. gigantica* infected and uninfected sheep

Antigen (µg/ml)									
F. hepatica					*F. gigantica*				
FhESP	B	N	P 1	P 2	FgESP	B	N	P 1	P 2
20	0.032	0.017	0.597	0.806	20	0.033	0.351	0.975	0.931
10	0.029	0.012	0.770	0.772	10	0.034	0.073	0.772	0.721
5	0.031	0.017	0.761	0.945	5	0.033	0.023	0.526	0.506
2.50	0.026	0.012	0.613	0.659	2.50	0.027	0.025	0.213	0.025
1.25	0.026	0.029	0.194	0.171	1.25	0.032	0.027	0.107	0.037
0.63	0.018	0.018	0.103	0.121	0.63	0.033	0.030	0.052	0.032
0.31	0.025	0.023	0.048	0.056	0.31	0.028	0.021	0.050	0.002
BBS	0.027	0.028	0.030	0.045	BBS	0.031	0.033	0.033	0.032
Serum Titrations									
F. hepatica					*F. gigantica*				
Serum	B	N	P 1	P 2	Serum	B	N	P 1	P 2
50	0.018	0.023	0.565	0.548	50	0.019	0.028	0.788	0.197
100	0.018	0.025	0.556	0.495	100	0.018	0.026	0.803	0.117
200	0.017	0.032	0.493	0.453	200	0.020	0.026	0.715	0.066
400	0.022	0.018	0.418	0.393	400	0.026	0.023	0.623	0.045
800	0.027	0.020	0.276	0.273	800	0.028	0.024	0.413	0.030
1600	0.021	0.017	0.147	0.108	1600	0.024	0.021	0.216	0.023
Monoclonal Antibody titration									
F. hepatica					*F. gigantica*				
McAb	B	N	P 1	P 2	McAb	B	N	P 1	P 2
10	0.036	0.100	1.084	1.011	10	0.030	0.060	0.826	0.251
20	0.035	0.082	0.975	1.003	20	0.036	0.041	0.776	0.222
40	0.032	0.059	0.717	0.813	40	0.031	0.042	0.581	0.148
80	0.033	0.034	0.374	0.356	80	0.034	0.034	0.310	0.159
160	0.029	0.019	0.204	0.258	160	0.029	0.028	0.138	0.066
320	0.029	0.018	0.089	0.160	320	0.033	0.042	0.062	0.037
Conjugate Titration									
F. hepatica					*F. gigantica*				
Conj.	B	N	P 1	P 2	Conj.	B	N	P 1	P 2
1000	0.027	0.028	0.808	0.156	1000	0.021	0.022	0.662	0.082
2000	0.015	0.020	0.504	0.085	2000	0.016	0.015	0.369	0.052
4000	0.021	0.017	0.279	0.059	4000	0.013	0.015	0.190	0.027
8000	0.014	0.015	0.154	0.038	8000	0.013	0.013	0.114	0.019
16000	0.011	0.009	0.078	0.020	16000	0.011	0.011	0.051	0.016
32000	0.007	0.013	0.050	0.013	32000	0.007	0.008	0.029	0.009

Appendix Table 4.48

Titration for total IgM showing the mean OD (450 nm) values obtained in *F. hepatica* and *F. gigantica* infected and uninfected sheep

Antigen (µg/ml)									
F. hepatica					*F. gigantica*				
FhESP	B	N	P 1	P 2	FgESP	B	N	P 1	P 2
20	0.017	0.311	1.090	0.919	20	0.023	0.495	0.774	0.924
10	0.013	0.314	0.986	0.903	10	0.015	0.433	0.766	0.887
5	0.016	0.279	0.944	0.874	5	0.010	0.366	0.690	0.926
2.50	0.012	0.273	0.828	0.731	2.50	0.021	0.205	0.475	0.856
1.25	0.008	0.221	0.684	0.694	1.25	0.013	0.317	0.617	0.892
0.63	0.011	0.124	0.491	0.220	0.63	0.008	0.326	0.312	0.326
Serum Titrations									
F. hepatica					*F. gigantica*				
Serum	B	N	P 1	P 2	Serum	B	N	P 1	P 2
50	0.029	0.640	0.954	0.889	50	0.028	0.580	0.878	0.749
100	0.021	0.430	0.903	0.784	100	0.022	0.420	0.739	0.617
200	0.019	0.290	0.823	0.629	200	0.024	0.297	0.581	0.453
400	0.018	0.159	0.672	0.456	400	0.017	0.350	0.393	0.350
800	0.019	0.099	0.545	0.309	800	0.013	0.208	0.272	0.208
1600	0.017	0.051	0.391	0.194	1600	0.015	0.105	0.156	0.105
Conjugate Titrations									
F. hepatica					*F. gigantica*				
Conj.	B	N	P 1	P 2	Conj.	B	N	P 1	P 2
1000	0.029	0.437	0.916	0.649	1000	0.033	0.383	0.555	0.610
2000	0.023	0.274	0.606	0.478	2000	0.032	0.228	0.306	0.360
4000	0.024	0.149	0.320	0.242	4000	0.016	0.125	0.148	0.178
8000	0.018	0.064	0.152	0.129	8000	0.020	0.069	0.084	0.105
16000	0.018	0.034	0.083	0.076	16000	0.012	0.026	0.034	0.053
32000	0.011	0.016	0.040	0.460	32000	0.014	0.017	0.013	0.013

Appendix Table 4.49

Titrations for IgG_2 showing the mean OD (450 nm) values obtained in *F. hepatica* and *F. gigantica* infected and uninfected sheep

Antigen (µg/ml)									
F. hepatica					*F. gigantica*				
FhESP	B	N	P 1	P 2	FgESP	B	N	P 1	P 2
20	0.023	0.037	0.306	0.115	20	0.033	0.030	0.196	0.133
10	0.019	0.032	0.479	0.081	10	0.034	0.052	0.120	0.163
5	0.023	0.027	0.470	0.254	5	0.033	0.002	0.074	0.048
2.50	0.017	0.042	0.322	0.168	2.50	0.027	0.004	0.061	0.037
1.25	0.017	0.029	0.003	-0.020	1.25	0.032	0.006	0.002	0.049
0.63	0.017	0.038	0.012	-0.070	0.63	0.033	0.009	0.040	0.044
0.31	0.012	0.023	-0.043	-0.035	0.31	0.028	0.000	0.038	0.014
BBS	0.011	0.028	-0.061	-0.046	BBS	0.031	0.012	0.021	0.044
Serum Titration									
F. hepatica					*F. gigantica*				
Serum	B	N	P 1	P 2	Serum	B	N	P 1	P 2
50	0.018	0.023	0.278	0.445	50	0.019	0.028	0.164	0.086
100	0.018	0.025	0.200	0.276	100	0.018	0.026	0.179	0.006
200	0.017	0.032	0.151	0.142	200	0.020	0.026	0.091	0.006
400	0.022	0.018	0.112	0.078	400	0.026	0.023	-0.001	-0.015
800	0.027	0.020	0.075	0.056	800	0.028	0.024	-0.011	-0.030
1600	0.021	0.017	0.072	0.038	1600	0.024	0.021	-0.028	-0.037
Monoclonal antibody titration									
F. hepatica					*F. gigantica*				
McAb	B	N	P 1	P 2	McAb	B	N	P 1	P 2
10	0.017	0.112	0.653	0.255	10	0.017	0.044	0.184	0.221
20	0.014	0.102	0.623	0.272	20	0.018	0.040	0.168	0.227
40	0.018	0.090	0.522	0.225	40	0.021	0.036	0.157	0.215
80	0.016	0.068	0.402	0.153	80	0.018	0.032	0.137	0.192
160	0.010	0.044	0.240	0.093	160	0.015	0.032	0.109	0.154
320	0.010	0.020	0.114	0.042	320	0.014	0.028	0.099	0.143
Conjugate Titration									
F. hepatica					*F. gigantica*				
Conj.	B	N	P 1	P 2	Conj.	B	N	P 1	P 2
1000	0.027	0.028	0.365	0.156	1000	0.021	0.022	0.512	0.082
2000	0.015	0.020	0.421	0.112	2000	0.016	0.015	0.369	0.052
4000	0.021	0.017	0.279	0.059	4000	0.013	0.015	0.312	0.027
8000	0.014	0.015	0.154	0.028	8000	0.013	0.013	0.114	0.019
16000	0.011	0.009	0.078	0.020	16000	0.011	0.011	0.051	0.016
32000	0.007	0.013	0.050	0.013	32000	0.007	0.008	0.029	0.009

Appendix Table 4.50

Titrations for IgA showing the mean OD (450 nm)values obtained in *F. hepatica* and *F. gigantica* infected and uninfected sheep

Antigen (µg/ml)									
F. hepatica					*F. gigantica*				
FhESP	B	N	P 1	P 2	FgESP	B	N	P 1	P 2
20	0.069	0.206	0.081	1.101	20	0.077	0.066	0.069	0.077
10	0.070	0.073	0.062	0.067	10	0.063	0.066	0.052	0.058
5	0.047	0.007	0.057	0.064	5	0.050	0.046	0.051	0.084
2.50	0.040	0.010	0.043	0.060	2.50	0.037	0.026	0.050	0.011
1.25	0.029	0.002	0.031	0.034	1.25	0.032	0.006	0.002	0.049
0.63	0.018	0.012	0.019	0.012	0.63	0.033	0.009	0.040	0.044
0.31	0.012	0.023	-0.043	-0.035	0.31	0.028	0.000	0.038	0.014
BBS	0.011	0.028	-0.061	-0.046	BBS	0.031	0.012	0.021	0.044
Serum titrations									
F. hepatica					*F. gigantica*				
Serum	B	N	P 1	P 2	Serum	B	N	P 1	P 2
50	0.018	0.023	0.278	0.445	50	0.019	0.028	0.164	0.086
100	0.018	0.025	0.200	0.276	100	0.018	0.026	0.179	0.006
200	0.017	0.032	0.151	0.142	200	0.020	0.026	0.091	0.006
400	0.022	0.018	0.112	0.078	400	0.026	0.023	-0.001	-0.015
800	0.027	0.020	0.075	0.056	800	0.028	0.024	-0.011	-0.030
1600	0.021	0.017	0.072	0.038	1600	0.024	0.021	-0.028	-0.037
Monoclonal antibody titrations									
F. hepatica					*F. gigantica*				
McAb	B	N	P 1	P 2	McAb	B	N	P 1	P 2
10	0.017	0.112	0.653	0.255	10	0.017	0.044	0.184	0.221
20	0.014	0.102	0.623	0.272	20	0.018	0.040	0.168	0.227
40	0.018	0.090	0.522	0.225	40	0.021	0.036	0.157	0.215
80	0.016	0.068	0.402	0.153	80	0.018	0.032	0.137	0.192
160	0.010	0.044	0.240	0.093	160	0.015	0.032	0.109	0.154
320	0.010	0.020	0.114	0.042	320	0.014	0.028	0.099	0.143
Conjugate Titration									
F. hepatica					*F. gigantica*				
Conj.	B	N	P 1	P 2	Conj.	B	N	P 1	P 2
1000	0.027	0.028	0.305	0.156	1000	0.021	0.022	0.212	0.082
2000	0.015	0.020	0.221	0.112	2000	0.016	0.015	0.169	0.052
4000	0.021	0.017	0.179	0.059	4000	0.013	0.015	0.112	0.027
8000	0.014	0.015	0.054	0.028	8000	0.013	0.013	0.114	0.019
16000	0.011	0.009	0.028	0.020	16000	0.011	0.011	0.051	0.016
32000	0.007	0.013	0.025	0.013	32000	0.007	0.008	0.029	0.009

Appendix Table 4.51

Titration for total Ig showing the mean OD (450 nm) values obtained in *F. hepatica* and *F. gigantica* infected and uninfected calves

Antigen (µg/ml) Titration									
F. hepatica					*F. gigantica*				
FhESP	B	N	P 1	P 2	FgESP	B	N	P 1	P 2
20	0.154	0.468	1.439	1.242	20	0.032	0.108	1.156	1.092
10	0.106	0.237	1.440	1.196	10	0.047	0.084	1.03	0.938
5	0.060	0.149	1.251	1.064	5	0.041	0.067	0.967	0.889
2.5	0.019	0.039	1.123	0.998	2.5	0.032	0.027	0.898	0.794
1.25	0.022	0.039	0.906	0.872	1.25	0.021	0.03	0.771	0.671
0.625	0.019	0.025	0.501	0.432	0.625	0.022	0.021	0.597	0.428
0.3125	0.016	0.016	0.339	0.158	0.3125	0.023	0.019	0.365	0.33
BBS	0.018	0.024	0.016	0.140	BBS	0.015	0.016	0.075	0.016
Serum Titrations									
F. hepatica					*F. gigantica*				
Serum	B	N	P 1	P 2	Serum	B	N	P 1	P 2
50	0.027	0.104	1.302	0.980	50	0.025	0.105	0.887	0.782
100	0.028	0.075	1.165	0.867	100	0.021	0.068	0.698	0.738
200	0.024	0.048	0.929	0.667	200	0.024	0.048	0.598	0.668
400	0.025	0.036	0.725	0.424	400	0.019	0.044	0.403	0.554
800	0.023	0.024	0.558	0.261	800	0.019	0.036	0.384	0.350
1600	0.024	0.025	0.415	0.155	1600	0.210	0.042	0.231	0.202
Conjugate titrations									
F. hepatica					*F. gigantica*				
Conj.	B	N	P 1	P 2	Conj.	B	N	P 1	P 2
1000	0.125	0.097	1.547	1.020	1000	0.02	0.08	1.249	1.111
2000	0.078	0.048	1.210	0.755	2000	0.014	0.033	0.874	0.764
4000	0.036	0.028	0.725	0.698	4000	0.012	0.014	0.537	0.388
8000	0.026	0.025	0.443	0.444	8000	0.01	0.01	0.238	0.17
16000	0.017	0.015	0.297	0.289	16000	0.009	0.004	0.0117	0.089
32000	0.013	0.014	0.147	0.125	32000	0.008	0.009	0.009	0.03

Appendix Table 4.52

Titration for IgG_1 showing the mean OD (450 nm) values obtained in *F. hepatica* and *F. gigantica* infected and uninfected calves

Antigen (µg/ml) Titration									
	F. hepatica					*F. gigantica*			
FhESP	B	N	P 1	P 2	FgESP	B	N	P 1	P 2
20	0.169	0.422	1.419	1.500	20	0.12	1.583	1.901	1.891
10	0.176	0.387	1.371	1.364	10	0.125	1.417	1.753	1.734
5	0.146	0.276	1.397	1.452	5	0.12	1.235	1.684	1.656
2.5	0.137	0.201	1.210	1.316	2.5	0.104	0.893	1.535	1.656
1.25	0.126	0.160	0.995	1.233	1.25	0.082	0.242	0.927	1.187
0.625	0.111	0.111	0.678	0.993	0.625	0.065	0.191	0.73	1.073
0.3125	0.120	0.098	0.443	0.811	0.3125	0.075	0.213	0.639	0.919
BBS	0.111	0.103	0.286	0.542	BBS	0.097	0.158	0.179	0.224
Serum titrations									
	F. hepatica					*F. gigantica*			
Serum	B	N	P 1	P 2	Serum	B	N	P 1	P 2
50	0.417	0.398	1.321	1.870	50	0.094	0.823	1.230	1.265
100	0.325	0.329	0.972	1.712	100	0.078	0.564	1.054	1.205
200	0.312	0.301	0.740	1.342	200	0.067	0.311	0.860	0.998
400	0.285	0.257	0.714	1.256	400	0.065	0.164	0.747	0.934
800	0.245	0.249	0.617	0.997	800	0.066	0.119	0.522	0.766
1600	0.218	0.224	0.548	0.682	1600	0.062	0.080	0.338	0.539
Conjugate titrations									
	F. hepatica					*F. gigantica*			
Conj.	B	N	P 1	P 2	Conj.	B	N	P 1	P 2
1000	0.483	0.590	1.551	1.690	1000	0.19	0.442	1.126	1.207
2000	0.285	0.418	1.423	1.563	2000	0.133	0.346	1.023	1.23
4000	0.183	0.264	1.364	1.532	4000	0.082	0.22	0.754	0.891
8000	0.137	0.159	1.288	1.501	8000	0.05	0.112	0.49	0.644
16000	0.060	0.117	1.157	1.347	16000	0.027	0.076	0.244	0.38
32000	0.037	0.054	0.959	1.117	32000	0.015	0.03	0.127	0.188

Appendix Table 4.53

Titrations for total IgM showing the mean OD (450 nm) values obtained in *F. hepatica* and *F. gigantica* infected and uninfected calves

Antigen (μg/ml) Titration									
F. hepatica					*F. gigantica*				
FhESP	B	N	P 1	P 2	FgESP	B	N	P 1	P 2
20	0.026	0.396	1.306	1.250	20	0.021	0.738	0.805	1.298
10	0.023	0.386	1.261	1.180	10	0.02	0.643	0.714	1.154
5	0.023	0.329	1.032	1.001	5	0.016	0.541	0.726	1.151
2.5	0.018	0.212	0.890	0.804	2.5	0.017	0.463	0.592	0.852
1.25	0.019	0.152	0.595	0.616	1.25	0.017	0.322	0.464	0.667
0.625	0.013	0.085	0.450	0.440	0.625	0.016	0.22	0.31	0.454
0.3125	0.017	0.062	0.363	0.364	0.3125	0.021	0.196	0.253	0.398
BBS	0.012	0.051	0.244	0.320	BBS	0.009	0.2	0.141	0.18
Serum titration									
F. hepatica					*F. gigantica*				
Serum	B	N	P 1	P 2	Serum	B	N	P 1	P 2
50	0.027	0.29	0.948	0.944	50	0.008	1.095	1.316	1.437
100	0.028	0.153	0.621	0.654	100	0.010	0.669	0.859	1.123
200	0.027	0.0743	0.362	0.371	200	0.008	0.353	0.596	0.765
400	0.027	0.0288	0.217	0.17	400	0.008	0.174	0.354	0.450
800	0.022	0.0173	0.127	0.104	800	0.012	0.102	0.182	0.254
1600	0.026	0.021	0.066	0.063	1600	0.013	0.041	0.088	0.139
Conjugate titrations									
F. hepatica					*F. gigantica*				
Conj.	B	N	P 1	P 2	Conj.	B	N	P 1	P 2
1000	0.031	1.432	1.503	0.238	1000	0.016	0.319	0.37	0.504
2000	0.035	1.283	1.332	0.190	2000	0.012	0.183	0.306	0.329
4000	0.026	1.006	1.145	0.143	4000	0.01	0.11	0.185	0.222
8000	0.015	0.830	0.748	0.081	8000	0.01	0.049	0.101	0.118
16000	0.014	0.484	0.518	0.037	16000	0.014	0.039	0.044	0.067
32000	0.024	0.275	0.307	0.016	32000	0.009	0.017	0.027	0.043

Appendix Table 4.54

Titrations for IgG_2 showing the mean OD (450 nm) values obtained in *F. hepatica* and *F. gigantica* infected and uninfected calves

Antigen (µg/ml) Titration									
F. hepatica					*F. gigantica*				
FhESP	B	N	P 1	P 2	FgESP	B	N	P 1	P 2
20	0.052	0.034	0.407	1.155	20	0.04	0.111	1.075	1.573
10	0.047	0.033	0.306	1.099	10	0.044	0.077	0.827	1.36
5	0.038	0.017	0.189	0.942	5	0.038	0.04	0.423	1.175
2.5	0.044	0.012	0.101	0.557	2.5	0.031	0.021	0.243	0.511
1.25	0.035	0.031	0.075	0.290	1.25	0.043	0.042	0.133	0.247
0.625	0.029	0.022	0.043	0.174	0.625	0.031	0.033	0.07	0.141
0.3125	0.030	0.027	0.040	0.077	0.3125	0.035	0.03	0.05	0.84
BBS	0.029	0.024	0.038	0.079	BBS	0.033	0.033	0.042	0.42
Serum titrations									
F. hepatica					*F. gigantica*				
Serum	B	N	P 1	P 2	Serum	B	N	P 1	P 2
50	0.076	0.093	0.386	1.320	50	0.054	0.272	1.870	1.782
100	0.079	0.083	0.244	1.206	100	0.035	0.130	0.922	1.709
200	0.089	0.082	0.126	0.672	200	0.035	0.074	0.670	1.279
400	0.095	0.077	0.106	0.290	400	0.036	0.038	0.393	1.005
800	0.090	0.074	0.076	0.220	800	0.031	0.028	0.251	0.721
1600	0.096	0.078	0.084	0.129	1600	0.038	0.022	0.151	0.378
Conjugate titrations									
F. hepatica					*F. gigantica*				
Conj.	B	N	P 1	P 2	Conj.	B	N	P 1	P 2
1000	0.058	0.033	0.184	0.702	1000	0.053	0.082	0.47	0.758
2000	0.034	0.090	0.126	0.505	2000	0.039	0.044	0.284	0.498
4000	0.020	-0.014	0.082	0.254	4000	0.028	0.021	0.158	0.284
8000	0.013	-0.021	0.032	0.156	8000	0.019	0.009	0.088	0.173
16000	0.009	-0.034	0.027	0.097	16000	0.016	0	0.04	0.097
32000	0.012	-0.033	0.023	0.048	32000	0.011	-0.008	0.01	0.037

Appendix Table 4.55

Titrations for IgA showing the mean OD (450 nm) values obtained in *F. hepatica* and *F. gigantica* infected and uninfected calves

Antigen (μg/ml) Titration									
F. hepatica					*F. gigantica*				
FhESP	B	N	P 1	P 2	FgESP	B	N	P 1	P 2
20	0.037	0.026	0.300	0.640	20	0.035	0.012	0.066	0.326
10	0.026	0.011	0.224	0.452	10	0.026	0	0.054	0.28
5	0.023	0.003	0.188	0.401	5	0.034	0.002	0.043	0.27
2.5	0.023	0.009	0.011	0.311	2.5	0.035	0.001	0.043	0.181
1.25	0.022	0.026	0.090	0.246	1.25	0.023	0.022	0.02	0.086
0.625	0.024	0.017	0.043	0.160	0.625	0.018	0.015	0.019	0.043
0.3125	0.022	0.017	0.033	0.193	0.3125	0.029	0.023	0.015	0.033
BBS	0.018	0.024	0.028	0.164	BBS	0.16	0.021	0.016	0.019
Serum Titrations									
F. hepatica					*F. gigantica*				
Serum	B	N	P 1	P 2	Serum	B	N	P 1	P 2
50	0.049	0.038	0.178	0.505	50	0.028	0.047	0.076	0.750
100	0.032	0.036	0.106	0.292	100	0.024	0.026	0.050	0.548
200	0.039	0.031	0.066	0.176	200	0.031	0.008	0.037	0.337
400	0.033	0.038	0.036	0.112	400	0.031	0.006	0.027	0.208
800	0.031	0.034	0.032	0.068	800	0.022	0.002	0.016	0.132
1600	0.037	0.041	0.033	0.051	1600	0.028	-0.001	0.013	0.100
Conjugate Titrations									
F. hepatica					*F. gigantica*				
Conj.	B	N	P 1	P 2	Conj.	B	N	P 1	P 2
1000	0.027	0.048	0.337	0.630	1000	0.026	0.066	0.095	0.662
2000	0.025	0.042	0.200	0.435	2000	0.02	0.038	0.05	0.542
4000	0.025	0.000	0.116	0.305	4000	0.015	0.023	0.015	0.327
8000	0.015	-0.002	0.064	0.180	8000	0.014	0.01	0.013	0.195
16000	0.013	-0.023	0.043	0.070	16000	0.014	0.005	-0.003	0.094
32000	0.013	-0.025	0.033	0.030	32000	0.013	-0.002	-0.006	0.055

Appendix Table 4

Titration for total Igand isotypes showing mean OD values obtained in *F. hepatica* and *F. gigantica* infected and uninfected sheep using Fh-cathepsin. as antigen

Appendix Table 4.75

Titration for total Ig showing the mean OD (450 nm)values obtained in *F. hepatica* and *F. gigantica* infected and uninfected sheep.

Cathepsin	B	N	P1	P2	Cathepsin	B	N	P1	P2
4	0.254	0.629	1.137	1.215	4	0.142	0.518	1.187	1.141
2	0.248	0.489	0.968	1.069	2	0.149	0.337	0.337	1.124
1	0.146	0.333	1.192	1.278	1	0.102	0.391	1.169	1.269
Serum	B	N	P1	P2	Serum	B	N	P1	P2
50	0.025	0.911	1.663	1.602	50	0.015	0.626	1.416	1.483
100	0.015	0.607	1.541	1.471	100	0.015	0.342	1.369	1.355
200	0.011	0.571	1.537	1.391	200	0.015	0.227	1.270	1.262
400	0.01	0.433	1.468	1.269	400	0.014	0.200	1.175	1.050
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.029	0.523	1.563	1.583	1000	0.015	0.424	0.102	1.459
2000	0.021	0.315	1.541	1.471	2000	0.015	0.227	1.270	1.262
4000	-0.032	0.254	1.552	1.466	4000	-0.04	0.185	1.150	1.150
8000	0.008	0.156	1.251	1.190	8000	0.006	0.056	0.797	1.262

Appendix Table 4.76

Titration for IgG_1 showing the mean OD (450nm) values obtained in *F. hepatica* and *F. gigantica* infected and uninfected sheep

Antigen (µg/ml) Titration IgG_1

Cathepsin	B	N	P1	P2	Cathepsin	B	N	P1	P2
4	0.031	0.439	1.917	1.204	4	0.041	0.059	0.365	1.692
2	0.055	0.361	1.422	1.003	2	0.039	0.040	0.280	1.107
1	0.043	0.265	0.979	0.750	1	0.027	0.031	0.245	0.543

Serum	B	N	P1	P2	Serum	B	N	P1	P2
50	0.051	0.090	0.691	0.502	50	0.050	0.064	0.179	0.368
100	0.049	0.040	0.602	0.419	100	0.043	0.051	0.165	0.317
200	0.065	0.087	0.230	0.282	200	0.023	0.034	0.160	0.259
400	0.070	0.060	0.135	0.171	400	0.020	0.029	0.068	0.090
McAb	B	N	P1	P2	McAb	B	N	P1	P2
20	0.049	0.186	0.602	0.419	20	0.043	0.051	0.165	0.317
40	0.068	0.194	0.480	0.371	40	0.060	0.057	0.135	0.168
80	0.081	0.136	0.211	0.224	80	0.071	0.075	0.110	0.149
160	0.087	0.102	0.151	0.158	160	0.074	0.071	0.094	0.117
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.055	0.140	1.619	0.278	1000	0.084	0.114	0.265	0.346
2000	0.044	0.110	1.216	0.222	2000	0.066	0.085	0.187	0.210
4000	0.041	0.062	0.849	0.129	4000	0.059	0.065	0.112	0.122
8000	0.039	0.050	0.490	0.087	8000	0.052	0.052	0.088	0.092
16000	0.045	0.038	0.287	0.067	16000	0.054	0.052	0.070	0.068
32000	0.048	0.043	0.153	0.059	32000	0.060	0.054	0.063	0.068

Appendix Table 4.77

Titrations for total IgM showing mean OD (450 nm) values obtained *in F. hepatica* and *F. gigantica* infected and uninfected sheep Fh-Cathepsin (µg/ml) as antigen

Antigen (µg/ml) Titration									
Cathepsin	B	N	P1	P2	Cathepsin	B	N	P1	P2
4	0.190	1.406	0.517	1.327	4	0.230	1.114	1.483	1.946
2	0.245	1.428	1.151	1.380	2	0.234	1.153	1.152	1.326
1	0.049	0.670	0.414	0.452	1	0.074	0.362	0.503	0.820

Serum	B	N	P1	P2	Serum	B	N	P1	P2
50	0.049	0.067	0.414	0.452	50	0.027	0.268	0.426	0.350
100	0.055	0.498	0.255	0.367	100	0.250	0.170	0.523	0.266
200	0.044	0.333	0.182	0.311	200	0.032	0.105	0.248	0.192
400	0.047	0.172	0.121	0.202	400	0.033	0.119	0.199	0.089

Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.100	0.075	1.150	0.965	1000	0.060	0.343	0.646	0.560
2000	0.035	0.389	0.681	0.570	2000	0.025	0.170	0.523	0.266
4000	0.028	0.162	0.251	0.127	4000	0.014	0.073	0.168	0.129
8000	0.032	0.101	0.189	0.154	8000	0.019	0.027	0.073	0.046

Appendix Table 4.78

Titrations for IgG_2 showing the mean OD (450 nm) values obtained in *F. hepatica* and *F. gigantica* infected and uninfected sheep Fh-Cathepsin (µg/ml) as antigen

Cathepsin	B	N	P1	P2	Cathepsin	B	N	P1	P2
4	0.052	0.084	0.195	0.088	4	0.042	0.050	0.050	0.040
2	0.048	0.067	0.138	0.073	2	0.041	0.059	0.052	0.050
1	0.051	0.073	0.086	0.072	1	0.051	0.057	0.059	0.044

Serum	B	N	P1	P2	Serum	B	N	P1	P2
50	0.123	0.229	0.203	0.149	50	0.100	0.129	0.129	0.114
100	0.054	0.075	0.088	0.074	100	0.051	0.057	0.059	0.044

McAb	B	N	P1	P2	McAb	B	N	P1	P2
10	0.108	0.285	0.250	0.165	10	0.097	0.152	0.131	0.124
20	0.123	0.229	0.203	0.149	20	0.100	0.129	0.129	0.114
40	0.102	0.102	0.138	0.122	40	0.084	0.137	0.103	0.106
80	0.089	0.103	0.124	0.122	80	0.079	0.09	0.09	0.091

Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.082	0.123	0.125	0.183	1000	0.084	0.019	0.112	0.117
2000	0.066	0.088	0.094	0.112	2000	0.070	0.074	0.076	0.079
4000	0.057	0.079	0.076	0.078	4000	0.057	0.065	0.063	0.064
8000	0.056	0.072	0.061	0.062	8000	0.058	0.054	0.052	0.055
16000	0.055	0.062	0.050	0.060	16000	0.051	0.048	0.053	0.052
32000	0.050	0.064	0.059	0.068	32000	0.063	0.051	0.058	0.060

Appendix Table 4.79

Titrations for IgA showing the mean OD (450 nm) values obtained in *F. hepatica* and *F. gigantica* infected and uninfected sheep

F. hepatica

Cathepsin	B	N	P1	P2
4	0.041	0.391	0.565	0.811
2	0.193	0.369	0.491	0.763
1	0.078	0.339	0.405	0.607

F.gigantica

Cathepsin	B	N	P1	P2
4	0.044	0.920	0.751	0.591
2	0.051	0.778	0.776	0.556
1	0.061	0.701	0.614	0.495

Serum	B	N	P1	P2
50	0.082	0.102	0.078	0.119
100	0.053	0.082	0.066	0.083

Serum	B	N	P1	P2
50	0.075	0.084	0.076	0.087
100	0.074	0.064	0.062	0.075

MeAb	B	N	P1	P2
10	0.079	0.100	0.067	0.079
20	0.082	0.102	0.078	0.083
40	0.079	0.099	0.075	0.087
80	0.075	0.095	0.074	0.082

MeAb	B	N	P1	P2
10	0.070	0.071	0.072	0.072
20	0.075	0.084	0.076	0.080
40	0.072	0.093	0.080	0.076
80	0.069	0.074	0.071	0.072

Conj.	B	N	P1	P2
1000	0.053	0.082	0.066	0.119
2000	0.039	0.050	0.042	0.068
4000	0.029	0.031	0.025	0.041
8000	0.028	0.026	0.018	0.030
16000	0.022	0.025	0.017	0.027
32000	0.026	0.027	0.018	0.026

Conj.	B	N	P1	P2
1000	0.074	0.064	0.062	0.087
2000	0.037	0.040	0.041	0.050
4000	0.033	0.029	0.029	0.037
8000	0.024	0.023	0.029	0.028
16000	0.023	0.021	0.019	0.021
32000	0.031	0.022	0.027	0.029

Table 4.80 Titration for total IgG showing the mean OD values obtained in *F. hepatica* and *F. gigantica* infected and uninfected Cattle, Fh-Cathepsin (µg/ml) as antigen

F. hepatica

Cathepsin	B	N	P1	P2
4	0.013	0.047	1.243	1.017
2	0.013	0.038	1.289	0.924
1	0.012	0.022	1.134	0.762

F. gigantica

Cathepsin	B	N	P1	P2
4	0.017	0.176	1.227	1.460
2	0.015	0.141	1.250	1.379
1	0.008	0.077	1.201	1.370

F. hepatica

Serum	B	N	P1	P2
50	0.001	0.038	1.460	1.144
100	-0.001	0.021	1.276	0.917
200	-0.001	0.015	1.063	0.669
400	-0.002	0.011	0.816	0.413
800	0.005	0.006	0.534	0.264
1600	0.000	0.012	0.390	0.175

F. gigantica

Serum	B	N	P1	P2
50	0.006	0.167	1.494	1.57
100	0.005	0.094	1.309	1.449
200	0.002	0.05	1.131	1.33
400	0.003	0.032	0.824	1.211
800	0.007	0.01	0.607	0.754
1600	0.003	0.01	0.292	0.661

F. hepatica

conj.	B	N	P1	P2
1000	0.034	0.105	1.332	1.086
2000	0.022	0.073	1.275	0.847
4000	0.011	0.05	1.053	0.637
8000	0.011	0.032	0.819	0.474
16000	0.005	0.029	0.57	0.28
32000	0.008	0.014	0.351	0.167

F. gigantica

conj.	B	N	P1	P2
1000	0.04	0.197	1.31	1.472
2000	0.022	0.108	1.192	1.272
4000	0.014	0.084	1.065	1.15
8000	0.012	0.06	0.812	0.952
16000	0.011	0.03	0.585	0.969
32000	0.015	0.021	0.355	0.415

Table 4.81:_Titration for IgG1 showing the mean OD values obtained in *F. hepatica* and *F. gigantica* infected and uninfected Cattle, Fh-Cathepsin (µg/ml) as antigen

F. hepatica						*F. gigantica*				
Cathepsin	B	N	P1	P2		Cathepsin	B	N	P1	P2
4	0.017	0.100	1.479	1.361		4	0.034	0.393	1.534	1.702
2	0.021	0.077	1.599	1.297		2	0.019	0.363	1.606	1.542
1	0.020	0.057	1.490	1.224		1	0.017	0.177	1.476	1.684
F. hepatica						*F. gigantica*				
Serum	B	N	P1	P2		Serum	B	N	P1	P2
50	0.014	0.119	1.609	1.461		50	0.011	0.407	1.628	1.766
100	0.01	0.094	1.411	1.378		100	0.012	0.306	1.608	1.742
200	0.014	0.048	1.497	1.213		200	0.022	0.124	1.524	1.676
400	0.017	0.032	1.347	0.911		400	0.013	0.08	1.331	1.522
800	0.009	0.023	1.119	0.666		800	0.012	0.045	1.099	1.444
1600	0.013	0.023	0.872	0.483		1600	0.014	0.039	0.78	1.105
F. hepatica						*F. gigantica*				
conj.	B	N	P1	P2		conj.	B	N	P1	P2
1000	0.046	0.092	1.897	1.230		1000	0.050	0.250	1.934	1.859
2000	0.026	0.056	1.669	0.894		2000	0.026	0.137	1.458	1.806
4000	0.022	0.040	1.288	0.650		4000	0.024	0.081	1.041	1.389
8000	0.013	0.017	0.851	0.348		8000	0.013	0.039	0.618	0.942
16000	0.011	0.015	0.542	0.193		16000	0.011	0.024	0.428	0.594
32000	0.010	0.011	0.394	0.104		32000	0.010	0.022	0.312	0.401

Table:4.82 Titration for IgM showing the mean OD values obtained in *F. hepatica* and *F. gigantica* infected and uninfected Cattle, Fh-Cathepsin (µg/ml) as antigen

F. hepatica						*F. gigantica*				
Antigen conc.	B	N	P1	P2		Antigen conc.	B	N	P1	P2
4	0.013	0.256	0.607	0.620		4	0.007	0.758	0.722	1.185
2	0.005	0.204	0.532	0.502		2	0.004	0.664	0.562	1.008
1	0.008	0.162	0.354	0.439		1	0.012	0.563	0.484	0.940
F. hepatica						*F. gigantica*				
Serum	B	N	P1	P2		Serum	B	N	P1	P2
100	0.069	0.198	0.741	0.512		100	0.042	0.578	0.912	0.857
200	0.049	0.109	0.562	0.457		200	0.035	0.511	0.803	0.749
400	0.035	0.099	0.279	0.31		400	0.041	0.365	0.451	0.384
800	0.022	0.072	0.221	0.124		800	0.025	0.219	0.354	0.325
1600	0.012	0.032	128	0.081		1600	0.033	0.102	0.234	0.241
3200	0.003	0.021	0.087	0.033		3200	0.054	0.088	0.142	0.149
F. hepatica						*F. gigantica*				
conj.	B	N	P1	P2		conj.	B	N	P1	P2
1000	0.029	0.287	0.807	0.605		1000	0.027	0.627	0.927	0.957
2000	0.013	0.176	0.477	0.367		2000	0.022	0.596	0.709	0.900
4000	0.011	0.103	0.358	0.205		4000	0.021	0.355	0.473	0.424
8000	0.009	0.067	0.199	0.154		8000	0.015	0.234	0.346	0.313
16000	0.06	0.037	0.108	0.063		16000	0.010	0.126	0.216	0.213
32000	0.003	0.028	0.062	0.046		32000	0.013	0.067	0.172	0.177

Table 4.83 Titration for IgG2 showing the mean OD values obtained in *F. hepatica* and *F. gigantica* infected and uninfected Cattle, Fh-Cathepsin (μg/ml) as antigen

F. hepatica						*F. gigantica*				
Cathepsin	B	N	P1	P2		Cathepsin	B	N	P1	P2
4	0.078	0.1	0.139	0.591		4	0.078	0.232	0.257	0.436
2	0.054	0.097	0.1	0.36		2	0.054	0.132	0.174	0.332
1	0.076	0.113	0.116	0.272		1	0.084	0.084	0.136	0.246
F. hepatica						*F. gigantica*				
Serum	B	N	P1	P2		Serum	B	N	P1	P2
50	0.051	0.074	0.137	0.658		50	0.044	0.112	0.285	0.735
100	0.03	0.035	0.055	0.171		100	0.031	0.042	0.104	0.208
200	0.018	0.018	0.032	0.126		200	0.018	0.032	0.048	0.071
400	0.019	0.008	0.021	0.061		400	0.018	0.028	0.029	0.038
800	0.014	0.009	0.016	0.039		800	0.010	0.010	0.018	0.018
1600	0.005	0.006	0.01	0.017		1600	0.009	0.005	0.014	0.011
F. hepatica						*F. gigantica*				
conj.	B	N	P1	P2		conj.	B	N	P1	P2
1000	0.084	0.171	0.330	1.034		1000	0.114	0.308	0.442	0.493
2000	0.041	0.088	0.194	0.709		2000	0.073	0.160	0.277	0.314
4000	0.035	0.057	0.114	0.330		4000	0.045	0.010	0.135	0.141
8000	0.025	0.032	0.062	0.191		8000	0.030	0.048	0.075	0.160
16000	0.024	0.021	0.340	0.103		16000	0.023	0.028	0.046	0.051
32000	0.015	0.019	0.270	0.061		32000	0.035	0.023	0.046	0.035

Table 4.84 Titration for IgA showing the mean OD values obtained in *F. hepatica* and *F. gigantica* infected and uninfected Cattle, Fh-Cathepsin (μg/ml) as antigen

F. hepatica						*F. gigantica*				
Cathepsin	B	N	P1	P2		Cathepsin	B	N	P1	P2
4	0.037	0.160	0.214	0.180		4	0.070	0.108	0.325	0.151
2	0.028	0.037	0.303	0.100		2	0.028	0.037	0.290	0.111
1	0.032	0.023	0.255	0.058		1	0.009	0.023	0.255	0.106
F. hepatica						*F. gigantica*				
Serum	B	N	P1	P2		Serum	B	N	P1	P2
50	0.034	0.044	0.385	0.109		50	0.034	0.044	0.325	0.193
100	0.029	0.025	0.203	0.156		100	0.029	0.035	0.187	0.156
200	0.030	0.022	0.167	0.070		200	0.024	0.026	0.081	0.119
400	0.024	0.028	0.088	0.024		400	0.019	0.017	0.041	0.089
800	0.011	0.015	0.024	0.014		800	0.014	0.008	0.024	0.065
1600	0.011	0.020	0.019	0.021		1600	0.009	0.001	0.029	0.054
F. hepatica						*F. gigantica*				
conj.	B	N	P1	P2		conj.	B	N	P1	P2
1000	0.034	0.044	0.185	0.090		1000	0.037	0.060	0.160	0.190
2000	0.029	0.025	0.103	0.056		2000	0.028	0.037	0.103	0.100
4000	0.030	0.040	0.067	0.035		4000	0.032	0.023	0.055	0.058
8000	0.024	0.028	0.038	0.024		8000	0.038	0.023	0.039	0.037
16000	0.011	0.015	0.024	0.014		16000	0.026	0.012	0.020	0.030
32000	0.011	0.020	0.019	0.021		32000	0.027	0.010	0.180	0.018

APPENDIX TABLE:4

ADJUSTED OPTICAL DENSITY (450 nm) VALUES OF
F. HEPATICA INFECTED SHEEP AND CATTLE

Experiment One (1): Adjusted values for *F. hepatica* (British and Peruvian strain) infected sheep (5, 6, 7, 9 and 10) and uninfected sheep (8)

Appendix Table 4.56

Adjusted total Ig values

WPI	SH5	SH6	SH7	SH8	SH9	SH10
-2	0.06	0.044	0.065	0.057	0.065	0.057
-1	0.063	0.035	-0.014	0.082	0.039	0.04
0	0.09	0.039	-0.014	0.044	0.07	0.065
1	0.073	0.127	0.025	0.063	0.604	0.379
2	0.372	0.48	0.068	0.082	0.472	0.549
3	0.463	0.656	0.228	0.038	0.611	0.432
4	0.514	0.597	0.324	0.032	0.628	0.421
5	0.554	0.746	0.441	0.001	0.78	0.581
6	0.665	0.72	0.681	0.051	0.768	0.74
7	0.739	0.824	0.759	-0.042	0.814	0.627
8	0.572	0.86	0.836	0.101	0.7	0.705
9	0.717	0.747	0.783	0.029	0.67	0.756
10	0.484	0.695	0.819	0.046	0.917	0.842
11	0.582	0.424	0.571	0.128	0.872	0.831
12	0.634	0.67	0.643	0.04	0.806	0.713
13		0.76	0.699	0.096	0.742	0.587
14		0.694	0.628	0.046	0.689	0.505
15		0.67	0.481	0.027	0.851	0.521
16		0.634	0.587	0.092	0.881	0.58
17		0.688	0.509	0.093	0.788	0.623

Adjusted IgG_1 values

WPI	SH. 5	SH. 6	SH. 7	SH. 8	SH. 9	SH. 10
-2	0.005	0.032	0.027	0.032	0.001	0.027
-1	0.004	-0.001	0.011	-0.001	-0.01	0.018
0	0.004	0.024	0.054	0.024	-0.005	0.043
1	0.006	0.056	0.062	0.056	0.46	0.378
2	0.152	0.73	0.067	0.027	0.677	0.473
3	0.499	0.564	0.223	0.011	0.606	0.622
4	0.629	0.844	0.424	0.054	0.597	0.624
5	0.66	0.984	0.505	0.062	0.643	0.62
6	0.7	1.009	0.68	0.067	0.667	0.667
7	0.706	1.029	0.747	0.027	0.7	0.68
8	0.636	1.087	0.678	0.018	0.783	0.769
9	0.593	1.017	0.723	0.043	0.817	0.841
10	0.379	1.026	0.789	0.032	0.862	0.84
11	0.42	0.905	0.757	-0.001	0.893	0.899
12	0.344	0.558	0.394	0.024	0.894	0.83
13		0.283	0.433	0.056	0.902	0.875
14		0.253	0.497	0.027	0.898	0.888
15		0.64	0.347	0.011	0.896	0.843
16		0.483	0.256	0.054	0.939	0.861
17		0.718	0.311	0.062	0.871	0.692

Appendix Table 4.57

Adjusted IgG_2 OD values

WPI	SH5	SH6	SH7	SH8	SH9	SH10
-2	0.04	0.008	0.002	0.004	0.019	0.008
-1	0.019	0.01	0	0.003	0.044	0.019
0	0.018	0.018	0.01	0.005	0.014	0.028
1	0.025	0.03	0.008	0.004	0.028	0.013
2	0.03	0.111	0.01	0.007	0.033	0.01
3	0.037	0.072	0.103	0.004	0.051	0.015
4	0.056	0.086	0.136	0	0.036	0.021
5	0.078	0.105	0.111	0.002	0.077	0.033
6	0.077	0.167	0.122	0.004	0.048	0.024
7	0.066	0.069	0.144	0.006	0.024	0.052
8	0.094	0.065	0.004	0.008	0.045	0.021
9	0.103	0.055	0.006	-0.007	0.042	0.049
10	0.057	0.056	0.019	0.004	0.033	0.033
11	0.071	0.01	0.027	0.007	0.03	0.038
12	0.076	0.007	0.007	0.006	0.015	0.021
13		0.03	0.008	0.003	0.036	0.044
14		0.052	0.049	0.002	0.019	0.016
15		0.02	0.027	0.004	0.048	0.033
16		0.01	0.032	0.008	0.038	0.02
17		0.009	0.031	0.003	-0.016	0.009

Adjusted IgM OD values

WPI	SH5	SH6	SH7	SH8	SH9	SH10
-2	-0.027	0.069	-0.062	-0.043	-0.029	0.058
-1	-0.055	-0.031	-0.069	-0.049	0.014	0.034
0	-0.046	-0.06	-0.054	-0.029	0.004	0.123
1	0.009	-0.017	-0.032	0.002	0.038	0.072
2	0.109	0.027	0.251	0.023	0.08	0.325
3	0.223	0.074	0.154	0.01	0.237	0.172
4	0.089	0.149	0.266	0.029	0.185	0.366
5	0.185	0.194	0.268	-0.022	0.391	0.465
6	0.228	0.308	0.414	-0.043	0.439	0.182
7	0.213	0.345	0.428	-0.028	0.411	0.231
8	0.229	0.091	0.394	-0.034	0.282	0.582
9	0.202	0.369	0.422	-0.017	0.528	0.371
10	0.043	0.385	0.347	-0.046	0.49	0.364
11	-0.002	0.423	0.398	-0.066	0.41	0.169
12	0.134	0.569	0.293	-0.009	0.475	0.428
13		0.305	0.218	-0.01	0.527	0.327
14		0.122	0.259	-0.046	0.18	0.232
15		0.113	0.114	0.014	0.252	0.18
16		0.198	0.194	-0.139	0.502	0.534
17		0.315	0.201	-0.025	0.254	0.178

Appendix Table 4.58

Adjusted IgA OD values

WPI	SH5	SH6	SH7	SH8	SH9	SH10
-2	0.036	0.067	0.048	0.084	0.031	0.005
-1	0.033	0.074	0.049	0.048	0.041	0.11
0	0.051	0.109	0.023	0.023	0.089	0.008
1	0.234	0.058	0.021	0.048	0.071	0.084
2	0.768	1.718	0.406	0.004	1.178	0.632
3	0.17	0.914	0.124	0.063	0.836	0.418
4	0.112	0.479	0.07	0.05	0.505	0.288
5	0.196	0.629	0.064	0.05	0.135	0.265
6	0.089	0.298	0.038	0.02	0.084	0.536
7	0.064	0.207	-0.005	0.053	0.158	0.031
8	0.066	0.386	0.006	0.111	0.217	0.031
9	0.07	0.297	-0.001	0.065	0.163	0.036
10	0.107	0.077	0.019	0.065	0.332	0.077
11	0.088	0.035	0.006	0.059	0.122	0.01
12	0.198	0.044	0.033	0.053	0.105	0.043
13		0.114	0.113	0.049	0.084	0.064
14		0.193	0.26	0.063	0.051	0.13
15		0.214	0.52	0.025	0.252	0.059
16		0.292	0.275	0.031	0.247	0.11
17		0.419	0.244	0.031	0.222	0.092

Experiment 2: Adjusted mean OD (450 nm) values for *F. hepatica* (British strain) infected sheep (24, 26, 28 and 30) and uninfected sheep (22 and 32)

Appendix Table 4.59

Adjusted total Ig OD values

WPI	SH24	SH26	SH28	SH30	SH22	SH32
-2	0.095	0.16	0.192	0.179	0.18	0.189
0	0.074	0.158	0.2	0.162	0.246	0.246
2	0.191	0.125	0.211	0.173	0.198	0.204
4	0.232	0.412	0.725	0.526	0.153	0.165
6	0.594	0.576	0.666	0.798	0.258	0.257
8	0.863	0.638	0.79	0.656	0.194	0.201
9	0.923	0.749	0.854	0.755	0.165	0.176
10	1.063	0.758	0.823	0.805	0.168	0.179
11	1.114	0.997	0.867	0.987	0.127	0.143
12	1.041	1.046	0.912	0.932	0.17	0.18
13	1.209	1.192	0.871	0.921	0.085	0.106
14	1.044	1.233	0.908	1.02	0.123	0.139
15	0.977	1.218	0.956	1.086	0.178	0.187
16	0.598	0.919	0.882	1.057	0.174	0.183
17	0.937	0.522	0.857	0.995	0.224	0.227
18	0.828	1.022	0.905	0.886	0.199	0.206
19	1.082	0.902	0.948	0.835	0.196	0.203
20	0.829	0.835	0.751	0.745	0.192	0.199
21	0.77	0.503	0.846	0.769	0.15	0.163
22	0.842	0.773	0.817	0.68	0.2	0.206

Adjusted IgG_1 OD values

WPI	SH. 24	SH. 26	SH. 28	SH. 30	SH. 22	SH. 32
-2	0.037	0.048	0.026	0.027	0.023	0.03
0	0.032	0.052	0.054	0.005	0.017	0.024
2	0.029	0.046	0.016	-0.008	0.07	0.037
4	0.022	0.031	0.012	0.005	0.032	0.039
6	0.199	0.089	0.038	0.069	0.029	0.036
8	0.579	0.132	0.032	0.638	0.04	0.047
9	0.774	0.958	0.484	0.849	0.011	0.018
10	0.74	1.125	0.75	0.987	0.023	0.03
11	1.065	1.232	0.86	1.095	0.034	0.041
12	1.168	1.142	1.164	1.233	0.053	0.06
13	1.135	1.243	1.286	1.25	0.036	0.043
14	1.182	1.264	1.482	1.257	0.026	0.033
15	1.189	1.206	1.378	1.482	0.023	0.03
16	1.2	1.253	1.034	1.602	0.027	0.034
17	1.189	1.264	1.198	1.673	0.031	0.038
18	1.126	1.229	0.744	1.314	0.026	0.033
19	1.069	1.088	0.772	1.166	0.023	0.02
20	1.084	1.114	0.654	1.19	0.016	0.033
21	1.121	0.83	0.65	1.097	0.018	0.025
22	1.098	0.554	0.958	1.137	0.026	0.033

Appendix Table 4.60

Adjusted IgM OD values

WPI	SH. 24	SH. 26	SH. 28	SH. 30	SH. 22	SH. 32
-2	0.129	0.17	0.096	0.124	0.124	0.093
0	0.119	0.192	0.268	0.104	0.14	0.149
2	0.331	0.465	0.641	0.592	0.026	0.208
4	0.737	0.507	0.925	0.495	0.088	0.076
6	0.652	0.555	0.901	0.495	0.02	0.148
8	0.525	0.759	0.816	0.998	0.074	0.064
9	0.608	0.698	0.811	0.87	0.066	0.025
10	0.512	0.711	0.786	0.856	0.039	0.043
11	0.487	0.555	0.914	0.737	0.048	0.071
12	0.419	0.443	0.884	0.983	0.065	0.071
13	0.508	0.717	0.797	0.722	0.06	0.04
14	0.589	0.608	0.868	0.909	0.153	0.088
15	0.665	0.56	0.69	0.592	0.101	0.066
16	0.621	0.415	0.749	0.567	0.184	0.046
17	0.479	0.466	0.788	0.559	0.169	0.039
18	0.476	0.585	0.596	0.488	0.17	0.126
19	0.679	0.58	0.686	0.457	0.155	0.185
20	0.308	0.311	0.556	0.403	0.157	0.152
21	0.291	0.548	0.548	0.643	0.141	0.106
22	0.208	0.49	0.59	0.445	0.156	0.1

Adjusted IgG_2 OD Values

WPI	SH. 24	SH. 26	SH. 28	SH. 30	SH. 22	SH. 32
-2	0.046	0.073	0.062	0.086	0.033	0.037
0	0.048	0.053	0.066	0.067	0.032	0.071
2	0.117	0.239	0.149	0.2	0.024	0.049
4	0.477	0.385	0.182	0.253	0.023	0.074
6	0.625	0.422	0.288	0.422	0.024	0.04
8	0.517	0.363	0.346	0.515	0.022	0.028
9	0.454	0.334	0.305	0.45	0.028	0.032
10	0.18	0.482	0.343	0.51	0.019	0.033
11	0.264	0.486	0.164	0.224	0.025	0.046
12	0.184	0.213	0.122	0.157	0.045	0.011
13	0.121	0.163	0.1	0.122	0.038	0.027
14	0.072	0.165	0.092	0.109	0.034	0.037
15	0.054	0.145	0.082	0.093	0.028	0.044
16	0.032	0.1	0.096	0.115	0.037	0.036
17	0.003	0.155	0.09	0.106	0.052	0.034
18	0.002	0.127	0.109	0.136	0.03	0.022
19	0.017	0.189	0.128	0.166	0.027	0.021
20	0.072	0.105	0.109	0.136	0.034	0.025
21	-0.011	0.218	0.062	0.061	0.022	0.023
22	0.001	0.153	0.084	0.096	0.039	0.017

Appendix Table 4.61

Adjusted IgA OD values

WPI	SH. 24	SH. 26	SH. 28	SH. 30	SH. 22	SH. 32
-2	-0.008	-0.021	-0.011	-0.005	-0.021	-0.012
0	0.016	-0.008	-0.009	-0.008	-0.035	-0.014
2	0.119	0.048	0.087	0.021	-0.019	-0.012
4	0.62	0.319	0.272	0.016	-0.021	-0.006
6	0.442	0.222	0.164	0.022	-0.023	-0.011
8	0.115	0.046	0.048	0.082	-0.013	-0.012
9	0.111	0.044	0.012	0.022	-0.015	-0.009
10	0.064	0.018	0.008	0.012	0	-0.014
11	0.088	0.031	-0.001	-0.004	0.011	-0.011
12	0.029	-0.001	0.023	-0.011	0.026	-0.016
13	0.146	0.063	0.036	-0.006	-0.01	-0.014
14	0.077	0.025	0.045	-0.005	-0.015	-0.011
15	0.127	0.052	0.06	-0.006	-0.013	-0.012
16	0.061	0.017	0.084	-0.002	-0.02	-0.014
17	0.1	0.038	0.171	0.009	-0.011	-0.008
18	0.117	0.047	0.218	0.052	-0.009	-0.011
19	0.171	0.076	0.301	0.098	-0.008	-0.006
20	0.436	0.219	0.288	0.055	0.001	-0.011
21	0.481	0.244	0.17	0.019	-0.009	-0.016
22	0.171	0.076	0.148	0.027	-0.004	-0.016

Experiment three (3): Adjusted mean OD (450 nm) values for *F. gigantica* (Kenyan strain) infected sheep and uninfected sheep

Appendix Table 4.62

Adjusted total Ig OD values

	Infected sheep								Uninfected Sheep						
WPI	SH.11	SH. 12	SH. 13	SH. 14	SH. 15	Mean	SDEV	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean	SDEV	SEM
-2	0.150	0.149	0.135	0.149	0.130	0.143	0.01	0.004	0.142	0.157	0.150	0.133	0.146	0.010	0.005
-1	0.091	0.223	0.109	0.164	0.108	0.139	0.05	0.024	0.303	0.091	0.186	0.105	0.171	0.097	0.049
0	0.093	0.175	0.169	0.165	0.287	0.178	0.07	0.031	0.291	0.176	0.185	0.142	0.199	0.064	0.032
1	0.255	0.214	0.150	0.207	0.349	0.235	0.07	0.033	0.326	0.231	0.289	0.165	0.253	0.070	0.035
2	0.570	0.309	0.453	0.377	0.748	0.491	0.17	0.077	0.189	0.254	0.239	0.064	0.187	0.086	0.043
3	0.734	0.869	0.925	1.094	1.159	0.956	0.17	0.077	0.253	0.028	0.191	0.022	0.124	0.117	0.058
4	0.871	1.371	1.169	1.265	1.251	1.185	0.19	0.085	0.264	0.215	0.079	0.158	0.179	0.080	0.040
5	0.841	1.330	1.220	1.250	1.463	1.221	0.23	0.104	0.153	0.008	0.088	0.069	0.080	0.060	0.030
6	1.002	1.384	1.444	1.142	1.541	1.303	0.22	0.100	0.202	0.265	0.166	0.172	0.201	0.045	0.023
7	1.038	1.533	1.532	1.173	1.430	1.341	0.22	0.100	0.118	0.304	0.150	0.235	0.202	0.084	0.042
8	1.027	1.497	1.404	1.247	1.687	1.372	0.25	0.112	0.177	0.106	0.186	0.112	0.145	0.042	0.021
9	1.484	1.399	1.688	1.330	1.434	1.467	0.14	0.061	0.174	0.215	0.185	0.158	0.183	0.024	0.012
10	1.355	1.487	1.459	1.276	1.332	1.382	0.09	0.040	0.209	0.018	0.289	0.029	0.136	0.134	0.067
11	1.464	1.586	1.454	1.324	1.339	1.433	0.11	0.048	0.380	0.142	0.239	0.016	0.194	0.154	0.077
12	1.383	1.541	1.646	1.939	1.258	1.553	0.26	0.117	0.236	0.127	0.191	0.120	0.169	0.055	0.028
13	1.471	1.614	1.512	1.364	1.264	1.445	0.14	0.060	0.299	0.293	0.079	0.192	0.216	0.104	0.052
14	1.402	1.634	1.522	1.379	1.127	1.413	0.19	0.085	0.254	0.041	0.088	0.028	0.103	0.104	0.052
15	1.463	1.681	1.295	1.320	1.272	1.406	0.17	0.076	0.241	0.254	0.166	0.052	0.178	0.093	0.046
16	1.521	1.652	1.535	1.207	1.145	1.412	0.22	0.100	0.184	0.041	0.150	0.017	0.098	0.082	0.041
17	1.076	1.704	1.326	1.259	1.340	1.341	0.23	0.102	0.177	0.060	0.186	0.091	0.129	0.063	0.031
18	1.055	1.553		1.350	1.272	1.308	0.21	0.103	0.242	0.192	0.185	0.148	0.192	0.039	0.019
19	1.042	1.566		1.253	1.247	1.277	0.22	0.108	0.189	0.111	0.289	0.114	0.176	0.084	0.042
20	0.978	1.587		1.227	1.232	1.256	0.25	0.125	0.253	0.199	0.239	0.252	0.236	0.025	0.013
21	1.084	1.440		1.236	1.127	1.222	0.16	0.080	0.196	0.358	0.191	0.317	0.266	0.085	0.042
22	1.041	1.404		1.273	1.272	1.248	0.15	0.076	0.209	0.170	0.079	0.277	0.184	0.083	0.041
23	1.015	1.563		1.176	1.145	1.225	0.24	0.118	0.225	0.161	0.088	0.135	0.152	0.057	0.029
24	1.031	1.348		1.283	0.903	1.141	0.21	0.105	0.175	0.212	0.166	0.157	0.178	0.024	0.012
25	1.023	1.081		1.153	1.051	1.077	0.06	0.028	0.178	0.158	0.103	0.134	0.143	0.032	0.016
26				1.264	1.081	1.173	0.13	0.092			0.092	0.187	0.140	0.067	0.048

Appendix Table 4.63

Adjusted IgG_1 OD values

	Infected sheep								Uninfected Sheep						
WPI	SH.11	SH. 12	SH. 13	SH. 14	SH. 15	Mean	SDEV	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean	SDEV	SEM
-2	0.105	0.069	0.048	0.038	0.046	0.061	0.03	0.012	0.054	0.060	0.057	0.051	0.056	0.004	0.002
0	0.245	0.258	0.013	0.023	0.051	0.118	0.12	0.055	0.024	0.036	0.049	0.027	0.034	0.011	0.006
2	1.752	0.298	0.495	1.538	0.183	0.853	0.74	0.329	0.034	0.044	0.037	0.066	0.045	0.014	0.007
4	1.883	0.696	1.127	1.303	0.708	1.143	0.49	0.219	0.098	0.055	0.068	0.086	0.077	0.019	0.010
6	1.669	1.048	1.221	1.367	0.918	1.245	0.29	0.131	0.068	0.071	0.064	0.062	0.066	0.004	0.002
8	1.652	1.387	1.800	1.816	1.046	1.540	0.33	0.146	0.054	0.060	0.073	0.051	0.060	0.010	0.005
9	1.728	1.821	1.948	1.772	1.128	1.679	0.32	0.143	0.089	0.088	0.071	0.079	0.082	0.008	0.004
10	1.812	1.831	1.757	1.772	1.106	1.656	0.31	0.138	0.084	0.027	0.049	0.105	0.066	0.035	0.018
11	1.739	1.826	1.859	1.191	1.060	1.535	0.38	0.170	0.056	0.062	0.075	0.053	0.062	0.010	0.005
12	1.897	1.760	1.494	1.183	1.176	1.502	0.33	0.147	0.035	0.045	0.058	0.036	0.044	0.011	0.005
13	1.755	1.782	1.497	1.596	1.143	1.555	0.26	0.115	0.058	0.063	0.076	0.054	0.063	0.010	0.005
14	1.863	1.431	1.369	1.283	1.033	1.396	0.3(	0.135	0.040	0.049	0.062	0.040	0.048	0.010	0.005
15	1.795	1.408		1.142	0.931	1.319	0.37	0.186	0.029	0.040	0.053	0.031	0.038	0.011	0.005
16	1.796	1.492		0.867	0.769	1.231	0.49	0.247	0.035	0.035	0.048	0.026	0.036	0.009	0.005
17	1.668	1.043		0.704	0.851	1.066	0.42	0.212	0.026	0.028	0.041	0.029	0.031	0.007	0.003
18	1.519	1.206		0.571	0.570	0.966	0.48	0.237	0.031	0.022	0.035	0.043	0.033	0.009	0.004
19	1.451	1.117		0.434	0.533	0.884	0.48	0.242	0.020	0.046	0.068	0.018	0.038	0.024	0.012
20	1.128	1.007		0.495	0.546	0.794	0.32	0.160	0.038	0.027	0.040	0.024	0.032	0.008	0.004
21	1.172	0.722		0.490	0.585	0.742	0.30	0.151	0.051	0.015	0.069	0.026	0.040	0.024	0.012
22	1.074	0.813		0.507	0.544	0.735	0.26	0.132	0.024	0.026	0.039	0.021	0.028	0.008	0.004

Appendix Table 4.64

Adjusted IgM OD values

	Infected sheep								Uninfected Sheep						
WPI	SH.11	SH. 12	SH. 13	SH. 14	SH. 15	Mean	SDEV	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean	SDEV	SEM
-2	0.106	0.050	0.048	0.078	0.043	0.065	0.03	0.012	0.045	0.102	0.041	0.049	0.059	0.029	0.014
0	0.051	0.075	0.054	0.066	0.024	0.054	0.02	0.009	0.056	0.118	0.058	0.051	0.071	0.032	0.016
2	0.258	0.193	0.226	0.228	0.306	0.242	0.04	0.019	0.013	0.003	0.077	0.021	0.029	0.033	0.017
4	0.287	0.090	0.227	0.317	0.236	0.231	0.09	0.039	0.019	0.023	0.062	0.014	0.030	0.022	0.011
6	0.278	0.322	0.446	0.342	0.174	0.312	0.10	0.044	0.019	0.064	0.062	0.010	0.039	0.028	0.014
8	0.294	0.203	0.348	0.267	0.191	0.261	0.07	0.029	0.074	0.087	0.084	0.063	0.077	0.011	0.005
9	0.287	0.142	0.319	0.450	0.202	0.280	0.12	0.053	0.044	0.046	0.040	0.043	0.043	0.003	0.001
10	0.241	0.133	0.338	0.225	0.364	0.260	0.09	0.042	0.026	0.033	0.037	0.058	0.039	0.014	0.007
11	0.260	0.104	0.346	0.318	0.339	0.273	0.10	0.045	0.026	0.030	0.013	0.086	0.039	0.032	0.016
12	0.354	0.114	0.282	0.530	0.276	0.311	0.15	0.067	0.005	0.069	0.065	0.071	0.053	0.032	0.016
13	0.324	0.225	0.268	0.534	0.383	0.347	0.12	0.054	0.068	0.054	0.048	0.071	0.060	0.011	0.006
14	0.376	0.317	0.291	0.492	0.308	0.357	0.08	0.037	0.060	0.068	0.064	0.054	0.062	0.006	0.003
15	0.405	0.269		0.528	0.256	0.365	0.13	0.064	0.075	0.075	0.041	0.138	0.082	0.041	0.020
16	0.346	0.221		0.464	0.251	0.321	0.11	0.055	0.068	0.035	0.040	0.060	0.051	0.016	0.008
17	0.337	0.234		0.444	0.205	0.305	0.11	0.054	0.039	0.073	0.080	0.056	0.062	0.018	0.009
18	0.326	0.180		0.282	0.223	0.253	0.06	0.032	0.036	0.035	0.028	0.063	0.041	0.015	0.008
19	0.283	0.218		0.366	0.199	0.267	0.08	0.038	0.042	0.044	0.037	0.053	0.044	0.007	0.003
20	0.182	0.170		0.323	0.194	0.217	0.07	0.036	0.045	0.024	0.125	0.082	0.069	0.044	0.022
21	0.295	0.117		0.195	0.095	0.176	0.09	0.045	0.071	0.084	0.080	0.062	0.074	0.010	0.005
22	0.126	0.100		0.231	0.318	0.194	0.10	0.050	0.012	0.002	0.076	0.021	0.028	0.033	0.017

Appendix Table 4.65

Adjusted IgG_2 OD values

	Infected sheep								Uninfected Sheep						
WPI	SH.11	SH. 12	SH. 13	SH. 14	SH. 15	Mean	SDEV	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean	SDEV	SEM
-2	0.032	0.032	0.034	0.036	0.036	0.034	0.002	0.001	0.034	0.038	0.043	0.032	0.037	0.005	0.002
0	0.045	0.031	0.036	0.023	0.043	0.036	0.009	0.004	0.019	0.026	0.028	0.014	0.022	0.006	0.003
2	0.027	0.032	0.038	0.029	0.004	0.026	0.013	0.006	0.035	0.038	0.044	0.034	0.038	0.005	0.002
4	0.034	0.025	0.033	0.031	0.018	0.028	0.007	0.003	0.021	0.028	0.030	0.017	0.024	0.006	0.003
6	0.040	0.020	0.022	0.034	0.032	0.030	0.008	0.004	0.011	0.020	0.020	0.005	0.014	0.007	0.004
8	0.026	0.032	0.042	0.044	0.002	0.029	0.017	0.008	0.049	0.049	0.058	0.050	0.052	0.004	0.002
9	0.033	0.016	0.017	0.031	0.016	0.023	0.009	0.004	0.055	0.053	0.064	0.058	0.058	0.005	0.002
10	0.034	0.007	0.028	0.023	0.020	0.022	0.010	0.005	0.009	0.019	0.018	0.002	0.012	0.008	0.004
11	0.030	0.018	0.019	0.032	0.009	0.022	0.009	0.004	0.016	0.024	0.025	0.011	0.019	0.007	0.003
12	0.052	0.012	0.027	0.027	0.059	0.035	0.020	0.009	0.008	0.018	0.017	0.001	0.011	0.008	0.004
13	0.047	0.032	0.016	0.020	0.049	0.033	0.015	0.007	0.012	0.021	0.021	0.006	0.015	0.007	0.004
14	0.052	0.027	0.017	0.023	0.059	0.036	0.019	0.008	0.014	0.023	0.023	0.008	0.017	0.007	0.004
15	0.028	0.012		0.012	0.005	0.014	0.010	0.005	0.003	0.014	0.012	0.019	0.012	0.007	0.003
16	0.031	0.021		0.012	0.013	0.019	0.009	0.004	0.025	0.031	0.034	0.022	0.028	0.005	0.003
17	0.062	0.025		0.023	0.081	0.048	0.029	0.014	0.031	0.035	0.040	0.029	0.034	0.005	0.002
18	0.042	0.016		0.031	0.036	0.031	0.011	0.006	0.046	0.047	0.055	0.047	0.049	0.004	0.002
19	0.033	0.018		0.010	0.016	0.019	0.010	0.005	0.030	0.035	0.039	0.028	0.033	0.005	0.002
20	0.031	0.005		0.022	0.013	0.018	0.011	0.006	0.061	0.058	0.070	0.065	0.064	0.005	0.003
21	0.027	0.019		0.018	0.004	0.017	0.010	0.005	0.036	0.039	0.045	0.035	0.039	0.005	0.002
22	0.034	0.014		0.029	0.018	0.024	0.009	0.005	0.032	0.036	0.041	0.030	0.035	0.005	0.002

Appendix Table 4.66

Adjusted IgA OD values

	Infected sheep								Uninfected Sheep						
WPI	SH.11	SH. 12	SH. 13	SH. 14	SH. 15	Mean	SDEV	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean	SDEV	SEM
-2	0.030	0.030	0.034	0.033	0.031	0.032	0.002	0.001	0.024	0.023	0.035	0.027	0.027	0.005	0.003
0	0.037	0.041	0.031	0.038	0.040	0.037	0.004	0.002	0.047	0.046	0.058	0.050	0.050	0.005	0.003
2	0.045	0.050	0.043	0.045	0.047	0.046	0.003	0.001	0.036	0.035	0.047	0.039	0.039	0.005	0.003
4	0.060	0.044	0.029	0.057	0.042	0.046	0.013	0.006	0.034	0.033	0.045	0.037	0.037	0.005	0.003
6	0.081	0.043	0.020	0.074	0.041	0.052	0.025	0.011	0.049	0.048	0.060	0.052	0.052	0.005	0.003
8	0.045	0.038	0.025	0.045	0.037	0.038	0.008	0.004	0.039	0.038	0.050	0.042	0.042	0.005	0.003
9	0.049	0.033	0.039	0.048	0.033	0.040	0.008	0.003	0.023	0.022	0.034	0.026	0.026	0.005	0.003
10	0.028	0.035	0.033	0.031	0.035	0.032	0.003	0.001	0.046	0.045	0.057	0.049	0.049	0.005	0.003
11	0.031	0.033	0.027	0.034	0.033	0.032	0.003	0.001	0.048	0.047	0.059	0.051	0.051	0.005	0.003
12	0.034	0.043	0.029	0.036	0.041	0.037	0.006	0.003	0.034	0.033	0.045	0.037	0.037	0.005	0.003
13	0.031	0.031	0.025	0.034	0.032	0.031	0.003	0.002	0.020	0.019	0.031	0.023	0.023	0.005	0.003
14	0.030	0.038	0.039	0.033	0.037	0.035	0.004	0.002	0.034	0.033	0.045	0.037	0.037	0.005	0.003
15	0.028	0.031		0.031	0.032	0.031	0.002	0 001	0.020	0.019	0.031	0.023	0.023	0.005	0.003
16	0.035	0.029		0.037	0.030	0.033	0.004	0.002	0.029	0.028	0.040	0.032	0.032	0.005	0.003
17	0.023	0.034		0.027	0.034	0.030	0.005	0.003	0.017	0.016	0.028	0.020	0.020	0.005	0.003
18	0.021	0.031		0.026	0.032	0.028	0.005	0.003	0.036	0.035	0.047	0.039	0.039	0.005	0.003
19	0.024	0.025		0.028	0.027	0.026	0.002	0.001	0.026	0.025	0.037	0.029	0.029	0.005	0.003
20	0.030	0.026		0.033	0.028	0.029	0.003	0.001	0.027	0.026	0.038	0.030	0.030	0.005	0.003
21	0.030	0.031		0.033	0.032	0.032	0.001	0.001	0.029	0.048	0.040	0.032	0.037	0.009	0.004
22	0.038	0.024		0.039	0.026	0.032	0.008	0.004	0.039	0.048	0.050	0.032	0.042	0.008	0.004

Experiment 4: Adjusted mean OD (450 nm) values for *F. gigantica* (Kenyan strain) infected sheep (23, 25, 27 and 29) and uninfected sheep (21 and 31)

Appendix Table 4.67

Adjusted total Ig OD values

WPI	SH.27	SH. 29	SH. 23	SH. 25	SH. 21	SH. 31
-2	0.205	0.205	0.201	0.211	0.209	0.207
-1	0.175	0.242	0.046	0.252	0.226	0.224
0	0.252	0.194	0.209	0.374	0.162	0.157
1	0.237	0.324	0.246	0.424	0.148	0.142
2	0.167	0.335	0.268	0.257	0.044	0.031
3	0.323	0.456	0.656	0.204	0.252	0.252
4	0.522	0.69	0.816	0.527	0.208	0.206
5	0.535	0.63	0.854	0.725	0.123	0.115
6	0.537	0.69	0.84	0.782	0.182	0.178
7	0.552	0.785	0.905	0.808	0.15	0.144
8	0.723	0.841	1.115	0.863	0.267	0.268
9	0.725	0.87	1.072	1.023	0.232	0.231
10	0.74	0.855	1.084	1.067	0.117	0.234
11	0.717	0.858	1.132	0.999	0.125	0.118
12	0.782	0.996	1.124	0.946	0.166	0.161
13	0.793	0.937	1.217	0.934	0.196	0.193
14	0.728	0.913	1.196	1.109	0.232	0.231
15	0.78	0.912	1.147	1.046	0.292	0.295
16	0.703	0.907		0.997	0.248	0.248
17	0.758	0.85		0.946	0.161	0.155
18	0.747	0.787			0.153	0.167
19	0.772	0.842			0.212	0.078
20	0.734	0.82			0.18	0.188
21	0.683	0.776			0.297	0.142
22	0.659	0.735			0.278	0.114

AdjustedIgG$_1$ OD values

WPI	SH. 21	SH. 23	SH. 25	SH. 27	SH. 29	SH. 31
-2	0.026	0.017	0.016	0.015	0.105	0.018
0	0.019	0.091	0.024	0.021	0.115	0.023
2	0.025	0.107	0.179	0.179	0.02	0.004
4	0.018	0.395	0.734	0.809	0.96	0.033
6	0.02	0.602	0.828	1.061	1.329	0.029
8	0.021	0.801	0.869	1.215	1.23	0.018
9	0.018	1.056	0.805	1.313	1.366	0.019
10	0.019	1.062	1.071	1.287	1.386	0.006
11	0.013	1.059	1.01	1.232	1.063	0.02
12	0.02	1.02	0.805	1.371	0.729	0.003
13	0.021	1.034	0.955	1.331	0.469	0.021
14	0.034	0.827	0.811	1.199	0.626	0.007
15	0.038	0.813	0.945	1.077	0.873	0.018
16	0.025		1.124	0.882	0.972	0.013
17	0.021			0.981	0.812	0.026
18	0.03			0.644	0.392	0.02
19	0.035			0.599	0.543	0.005
20	0.041			0.615	0.41	0.025
21	0.029			0.662	0.297	0.013
22	0.028			0.612	0.162	0.004

Appendix Table 4.68

Adjusted IgM OD values

WPI	SH. 21	SH. 23	SH. 25	SH. 27	SH. 29	SH. 31
-2	0.152	0.108	0.131	0.178	0.142	0.103
0	0.134	0.101	0.116	0.166	0.112	0.112
2	0.114	0.121	0.118	0.433	0.376	0.085
4	0.11	0.515	0.453	0.511	0.506	0.133
6	0.064	0.297	0.383	0.357	0.666	0.088
8	0.09	0.293	0.395	0.306	0.4	0.076
9	0.11	0.426	0.414	0.409	0.499	0.046
10	0.09	0.417	0.756	0.37	0.671	0.03
11	0.082	0.304	0.511	0.39	0.459	0.079
12	0.134	0.325	0.34	0.399	0.515	0.028
13	0.136	0.332	0.39	0.556	0.448	0.107
14	0.066	0.384	0.4	0.624	0.655	0.13
15	0.05	0.466	0.534	0.642	0.547	0.142
16	0.136		0.445	0.538	0.918	0.07
17	0.138			0.573	0.47	0.122
18	0.074			0.455	0.347	0.058
19	0.13			0.419	0.432	0.169
20	0.054			0.409	0.326	0.12
21	0.04			0.298	0.205	0.049
22	0.158			0.231	0.167	0.113

Adjusted IgG_2 OD values

WPI	SH. 21	SH. 23	SH. 25	SH. 27	SH. 29	SH. 31
-2	0.033	0.037	0.029	0.042	0.032	0.038
0	0.027	0.025	0.01	0.024	0.02	0.034
2	0.03	0.038	0.022	0.035	0.033	0.036
4	0.026	0.026	0.029	0.042	0.022	0.033
6	0.023	0.018	0.001	0.014	0.014	0.03
8	0.028	0.049	0.004	0.018	0.044	0.034
9	0.027	0.054	0.002	0.016	0.049	0.034
10	0.029	0.017	0.01	0.023	0.012	0.035
11	0.029	0.022	0.01	0.023	0.018	0.035
12	0.019	0.016	0.011	0.025	0.011	0.027
13	0.033	0.019	0.02	0.034	0.014	0.038
14	0.027	0.021	0.014	0.028	0.016	0.034
15	0.017	0.012	0.009	0.022	0.007	0.026
16	0.019	0.03	0.01	0.024	0.025	0.027
17	0.021	0.034	0.014	0.027	0.03	0.029
18	0.023	0.046	0.006	0.02	0.042	0.03
19	0.037	0.034	0.005	0.018	0.029	0.042
20	0.026	0.058	0.01	0.024	0.054	0.033
21	0.02	0.038	0.002	0.016	0.034	0.028
22	0.033	0.035	-0.009	0.005	0.03	0.038

Appendix Table 4.69

Adjusted IgA OD values

WPI	SH. 21	SH. 23	SH. 25	SH. 27	SH. 29	SH. 31
-2	0.024	0.03	0.034	0.003	0.127	0.035
0	0.047	0.069	0.054	0.042	0.166	0.058
2	0.036	0.067	0.083	0.04	0.164	0.047
4	0.034	0.072	0.114	0.045	0.169	0.045
6	0.049	0.758	0.278	0.731	0.855	0.06
8	0.039	0.273	0.405	0.246	0.37	0.05
9	0.023	0.243	0.593	0.216	0.34	0.034
10	0.046	0.151	0.691	0.124	0.248	0.057
11	0.048	0.113	0.061	0.086	0.21	0.059
12	0.034	0.086	0.128	0.059	0.183	0.045
13	0.02	0.11	0.244	0.083	0.207	0.031
14	0.034	0.093	0.224	0.066	0.19	0.045
15	0.02	0.273	0.27	0.246	0.37	0.031
16	0.029	0.4		0.373	0.257	0.04
17	0.017			0.284	0.238	0.028
18	0.036			0.264	0.295	0.047
19	0.026			0.31	0.284	0.037
20	0.027			0.47	0.187	0.038
21	0.049			0.294	0.318	0.06
22	0.049			0.31	0.434	0.06

Experiment 5 and 6: Calves (14c, 15c, 23c, 34c and 45c) infected with *F. hepatica* and uninfected calf (26c) and F. gigantica infected calves (22,23 and 24) and uninfected calf (26)

Adjusted mean OD (450 nm) values in response to Fh-E/S and Fh-E/S

Appendix Table 4.70

Adjusted total Ig OD values

	F. hepatica							F. Gigntica			
WPI	14c	15c	23c	26c	34c	45c	WPI	calf 22	calf 23	calf 24	calf 26
-27	0.012	0.037	0.081	0.081			0	0.013	-0.007	0.011	-0.001
-26	0.030	0.010	0.078	0.043			1	0.024	-0.019	-0.021	-0.033
-25	0.022	0.016	0.012	0.026			2	0.087	0.085	0.097	0.009
-24	0.039	0.003	0.012	0.020			3	0.034	0.092	0.063	0.035
-23	0.081	0.011	0.033	0.026			4	0.103	0.149	0.292	-0.079
-22	0.171	-0.075	0.006	-0.017			5	0.426	0.606	0.734	-0.125
-21	0.396	0.422	-0.007	-0.005			6	0.791	0.766	0.785	0.028
-20	0.592	0.496	-0.012	-0.004			7	0.897	0.741	1.055	-0.073
-19	0.717	1.221	-0.023	-0.025			8	1.014	0.697	0.942	0.043
-18	1.008	0.980	-0.020	-0.017			9	0.997	0.684	1.130	0.114
-17	1.012	1.645	0.005	-0.001			10	0.915	0.979	0.995	-0.025
-16	1.358	1.243	-0.001	0.004			11	1.032	0.940	0.800	0.028
-15	0.816	0.965	0.026	0.038			12	0.906	0.728	0.969	-0.118
-14	0.691	1.409	0.060	0.020			13	0.941	0.848	1.013	-0.133
-13	0.593	1.458	0.046	0.019			14	0.811	0.939	1.160	-0.015
-12	0.571	1.259	-0.005	0.003			15	0.807	0.995	0.997	0.065
-11	0.560	0.754	0.399	0.001			16	1.006	0.963	1.111	-0.119
-10	0.818	0.660	0.961	-0.017			17	1.008	1.016	0.982	-0.154
-9	0.887	1.284	1.052	-0.016			18	0.996	1.035	1.086	-0.131
-8	1.088	1.131	0.957	-0.018			19	0.980	0.856	1.186	-0.048
-7	1.232	1.079	1.199	-0.009			20	0.842	0.832	1.088	0.016
-6	1.046	1.179	1.285	0.021			21	0.914	0.955	1.116	-0.023
-5	1.369	1.121	1.190	0.035			22	0.844	0.937	0.935	0.133
-4	1.192	1.217	1.161	0.053			23	0.970	0.974	0.971	0.205
-3	1.140	1.269	1.173	0.014			24	0.933	1.066	1.226	0.160
-2	1.150	1.538	1.095	0.014			25	0.824	1.150	0.883	0.001
-1	0.956	1.557	1.169	0.023	0.066	0.007	26	0.914	1.104	1.056	0.026
0	0.998	1.652	1.177	0.053	0.093	0.087	27	0.736	1.005	1.011	0.000
1	0.984	1.427	1.148	0.015	0.193	0.183	28	0.889	0.991	0.891	0.060
2	0.932	1.647	1.039	0.070	0.242	0.198	29	0.906	1.075	1.071	0.096
3	0.958	1.646	1.235	0.038	0.306	0.140	30	0.870	0.993	0.944	0.095
4	1.195	1.680	1.067	0.014	0.385	0.368	31	0.788	1.112	1.063	0.199
5	1.164	1.688	1.004	0.006	0.479	0.948	32	0.935	1.107	0.986	0.149
6	1.398	1.759	0.857	0.017	0.949	1.066	33	0.936	1.098	0.969	0.101
7	1.261	1.706	0.743		0.841	1.402	34	0.825	1.113	1.018	-0.011
8	1.062	1.677	0.982		1.699	1.504		0.709	1.143	1.256	-0.003
9	1.132	1.679	0.645		0.657	1.079		0.672			0.076
10	0.995	1.465	0.891		1.480	1.135					
11	1.139	1.715	1.120			1.340					

Appendix Table 4.71
Adjusted IgG_1 OD values

	F. hepatica							F. Gigntica			
WPI	14c	15c	23c	26c	34c	45c	WPI	calf 22	calf 23	calf 24	calf 26
-27	0.146	-0.009	0.140	0.030			-2	0.088	0.076	0.056	0.074
-26	0.024	0.088	0.111	0.007			-1	-0.04	0.006	0.004	0.005
-25	0.024	0.007	0.085	0.011			0	-0.04	-0.023	-0.028	0.032
-24	0.028	-0.077	0.031	-0.013			1	0.00	0.156	0.024	0.115
-23	0.053	-0.039	0.004	0.024			2	-0.02	0.128	-0.223	-0.024
-22	0.067	0.071	-0.036	-0.046			3	0.37	0.292	0.573	0.082
-21	0.274	0.608	-0.014	-0.082			4	0.591	0.835	0.800	0.056
-20	0.739	1.014	-0.030	-0.104			5	0.557	0.830	0.800	0.120
-19	0.968	1.223	-0.040	-0.064			6	0.598	0.746	0.518	0.074
-18	1.185	1.326	-0.061	-0.107			7	0.536	0.695	0.661	0.052
-17	1.319	1.404	-0.018	-0.054			8	0.650	0.811	0.789	0.108
-16	1.269	1.290	0.225	-0.062			9	0.627	0.807	0.741	0.098
-15	1.302	1.324	0.185	-0.084			10	0.453	0.737	0.616	-0.014
-14	1.327	1.404	0.284	-0.084			11	0.369	0.789	0.700	0.128
-13	1.211	1.447	0.404	-0.055			12	0.320	0.700	0.707	0.037
-12	1.211	1.069	0.933	-0.069			13	0.656	0.988	0.695	0.100
-11	1.090	1.103	1.036	-0.082			14	0.732	0.986	0.825	0.083
-10	1.031	1.076	1.159	0.019			15	0.647	0.801	0.798	0.065
-9	1.237	1.234	1.185	-0.036			16	0.641	0.746	0.932	0.075
-8	1.109	1.207	1.254	-0.028			17	0.501	0.754	1.030	0.109
-7	1.369	1.139	1.259	0.004			18	0.454	0.928	0.522	0.116
-6	1.287	1.297	1.264	0.005			19	0.643	0.826	0.620	0.088
-5	1.299	1.248	1.251	-0.054			20	0.479	0.607	0.664	0.111
-4	1.305	1.207	1.266	-0.007			21	0.665	0.683	0.785	0.074
-3	1.324	1.213	1.304	0.011			22	0.492	0.890	0.729	0.103
-2	1.287	1.077	1.269	0.009			23	0.622	0.991	0.395	0.014
-1	1.170	1.220	1.161	0.061			24	0.536	1.037	0.434	0.102
0	1.046	0.985	1.268	-0.014	0.033	0.127	25	0.491	0.873	0.437	0.074
1	1.232	1.284	1.143	-0.036	0.400	0.609	26	0.467	0.743	0.451	0.154
2	1.206	1.482	1.123	0.003	0.782	0.784	27	0.584	0.692	0.493	0.099
3	1.108	1.484	1.226	-0.035	0.698	0.494	28	0.456	1.141	0.214	0.104
4	1.030	1.508	1.278	0.013	0.637	0.453	29	0.503	0.904	0.226	0.004
5	1.097	1.488	1.131	-0.032	0.833	0.876	30	0.582	0.917	0.517	-0.025
6	0.967	1.533	1.059	-0.069	1.080	1.275	31	0.553	0.833	0.310	0.086
7	1.287	1.539	1.169		1.377	1.503	32	0.454	0.771	0.223	0.126
8	1.160	1.548	0.969		1.374	1.451	33	0.447	0.826	0.164	0.074
9	0.995	1.533	1.100		1.525	1.473					
10	0.959	1.487	1.159		1.479	1.594					
11	1.007	1.501	1.021		1.475	1.584					
12	1.011	1.444	0.886			1.551					

Appendix Table 4.72

Adjusted IgM OD values values

F. hepatica							*F. Gigntica*				
WPI	14c	15c	23c	26c	34c	45c	WPI	calf 22	calf 23	calf 24	calf 26
-27	0.045	0.032	0.018	0.038			-2	0.040	0.088	0.080	0.051
-26	0.132	-0.01	0.013	0.005			-1	0.020	-0.074	0.002	0.042
-25	0.177	-0.01	0.098	0.014			0	0.061	0.054	0.122	0.048
-24	0.097	0.065	0.137	-0.020			1	0.111	-0.031	0.050	0.132
-23	0.895	0.566	0.052	0.020			2	0.051	0.240	0.256	0.047
-22	1.104	0.681	0.129	-0.058			3	0.370	0.610	0.273	-0.003
-21	1.165	0.670	0.104	-0.105			4	0.495	0.504	0.467	0.055
-20	1.068	0.522	0.054	-0.139			5	0.286	0.515	0.319	0.070
-19	0.561	0.996	0.017	-0.083			6	0.331	0.568	0.243	0.011
-18	0.543	0.759	0.077	-0.140			7	0.126	0.336	0.030	0.066
-17	0.734	0.739	0.123	-0.064			8	0.102	0.427	0.005	0.039
-16	0.531	0.741	0.098	-0.080			9	0.323	0.583	0.141	-0.048
-15	0.668	0.913	0.137	-0.106			10	0.383	0.548	0.167	0.084
-14	0.415	0.650	0.373	-0.111			11	0.624	0.517	0.533	0.005
-13	0.483	0.592	1.266	-0.073			12	0.622	0.512	0.543	0.034
-12	0.402	0.807	1.237	-0.096			13	0.442	0.327	0.549	-0.067
-11	0.538	1.314	0.852	-0.121			14	0.462	0.408	0.590	-0.013
-10	0.635	0.932	0.628	0.010			15	0.289	0.341	0.480	0.057
-9	0.818	0.815	0.569	-0.056			16	0.176	0.522	0.621	0.059
-8	0.837	1.165	0.740	-0.024			17	0.266	0.440	0.762	0.015
-7	0.965	0.818	0.588	0.004			18	0.400	0.336	0.585	0.000
-6	0.817	0.755	0.526	0.004			19	0.461	0.320	0.780	-0.018
-5	0.779	0.657	0.427	-0.070			20	0.156	0.284	0.788	0.060
-4	0.777	0.539	0.468	-0.005			21	0.222	0.415	0.641	0.049
-3	0.678	0.930	0.652	0.011			22	0.113	0.707	0.484	0.044
-2	0.176	0.716	0.631	0.005			23	0.304	0.435	0.546	0.007
-1	0.746	0.614	0.572	0.071			24	0.284	0.361	0.760	0.001
0	0.672	0.640	0.788	-0.024	0.050	0.017	25	0.207	0.435	0.541	0.098
1	0.779	0.446	0.500	-0.053	0.250	0.059	26	0.061	0.381	0.653	-0.008
2	0.470	0.507	0.688	0.004	0.293	0.235	27	0.186	0.363	0.561	0.016
3	0.708	0.487	0.712	-0.050	0.277	0.289	28	0.245	0.050	0.507	0.030
4	0.580	0.562	0.775	0.015	0.452	0.293	29	0.135	0.177	0.572	0.035
5	0.738	0.462	0.752	-0.046	0.551	0.685	30	0.273	0.269	0.534	0.045
6	0.550	0.334	0.844	-0.093	0.911	0.950	31	0.098	0.295	0.483	0.063
7	0.651	0.441	0.871		0.689	1.306	32	0.069	0.010	0.428	-0.040
8	0.670	0.542	0.718		0.706	1.120	33	0.129	0.247	0.534	0.036
9	0.586	0.408	0.695		0.659	0.792					
10	0.639	0.578	0.630		0.572	0.838					
11	0.838	0.528	0.561		0.637	0.850					
12	0.907	0.344	0.718			0.903					

Appendix Table 4.73
Adjusted IgG_2 OD values

F. hepatica							*F. gigantica*				
WPI	14c	15c	23c	26c	34c	45c	WPI	calf 22	calf 23	calf 24	calf 26
-27	0.020	0.009	0.019	0.017			-2	0.010	0.052	0.005	0.012
-26	0.022	0.014	0.023	0.020			-1	0.007	0.037	0.007	0.023
-25	0.025	0.024	0.037	0.020			0	0.049	0.079	0.013	0.036
-24	0.029	0.016	0.042	0.029			1	0.105	0.091	0.028	0.026
-23	0.025	0.009	0.033	0.017			2	0.006	0.147	0.009	-0.081
-22	-0.01	-0.012	0.012	-0.014			3	0.00	0.070	0.029	0.042
-21	0.00	0.028	-0.003	-0.011			4	0.042	0.085	0.104	0.083
-20	0.011	0.074	-0.001	-0.017			5	0.026	0.040	0.166	0.070
-19	0.024	0.128	0.011	-0.011			6	0.078	0.101	0.225	0.019
-18	0.069	0.195	0.001	-0.002			7	0.087	0.048	0.270	0.004
-17	0.086	0.646	0.035	0.033			8	0.153	0.104	0.226	0.045
-16	0.189	0.593	0.041	0.029			9	0.131	0.087	0.221	0.056
-15	0.144	0.117	0.034	0.032			10	0.076	0.419	0.186	0.055
-14	0.575	0.419	0.032	0.024			11	0.046	0.412	0.222	0.036
-13	0.159	0.382	0.042	0.025			12	0.047	0.489	0.094	0.025
-12	0.118	0.407	0.120	-0.005			13	0.092	0.247	0.231	0.035
-11	0.078	0.315	0.271	-0.006			14	0.222	0.129	0.313	0.064
-10	0.094	0.462	0.351	-0.009			15	0.204	0.392	0.273	0.040
-9	0.223	0.723	0.378	-0.002			16	0.189	0.312	0.304	0.023
-8	0.465	0.481	0.475	-0.003			17	0.168	0.476	0.005	-0.010
-7	0.828	0.546	0.617	0.020			18	0.143	0.343	0.229	-0.009
-6	0.795	0.771	0.745	0.025			19	0.138	0.254	0.251	0.041
-5	0.891	0.977	0.647	0.019			20	0.139	0.350	0.209	0.023
-4	0.963	0.905	0.745	0.036			21	0.227	0.334	0.236	0.028
-3	0.997	1.092	0.580	0.056			22	0.147	0.467	0.325	0.000
-2	0.844	1.302	0.512	0.053			23	0.242	0.407	0.181	0.024
-1	1.175	1.354	0.487	0.052			24	0.221	0.562	0.290	0.037
0	1.004	1.474	0.805	0.046	0.014	0.012	25	0.151	0.489	0.239	0.053
1	1.050	1.555	0.667	0.041	0.017	0.027	26	0.137	0.445	0.277	0.001
2	0.910	1.452	0.703	0.057	0.035	0.043	27	0.174	0.595	0.332	0.030
3	0.992	1.288	0.493	0.039	0.050	0.041	28	0.162	0.702	0.287	0.025
4	0.907	1.453	0.661	0.045	0.061	0.026	29	0.130	0.508	0.316	0.072
5	1.133	1.409	0.634	0.040	0.067	0.817	30	0.168	0.876	0.315	0.026
6	0.942	1.212	0.646	0.024	0.111	0.057	31	0.168	0.600	0.313	0.052
7	0.848	0.992	0.821		0.230	0.164	32	0.128	0.782	0.297	0.013
8	1.018	1.139	1.354		0.580	0.312	33	0.093	0.765	0.301	0.002
9	0.906	1.080	1.352		0.867	0.409					
10	0.835	1.022	1.207		0.622	0.419					
11	0.915	0.882	1.418		0.778	0.479					
12	0.885	0.744	1.550			0.522					

Appendix Table 4.74

Adjusted IgA Values values

	F. hepatica							F. Gigntica				
WPI	14c	15c	23c	26c	34c	45c	WPI	calf 22	calf 23	calf 24	calf 26
-27	0.010	0.029	-0.040	0.017			-2	0.049	0.042	-0.012	0.018
-26	0.007	0.027	-0.018	0.008			-1	0.018	0.012	-0.006	0.008
-25	0.009	0.033	0.020	0.019			0	0.098	0.089	-0.010	0.038
-24	0.003	0.027	0.024	0.020			1	0.056	0.048	0.009	0.043
-23	0.028	0.055	-0.015	0.047			2	0.044	0.037	-0.006	0.057
-22	0.073	0.048	-0.061	0.000			3	0.074	0.066	0.053	0.031
-21	0.059	0.029	-0.064	0.012			4	0.074	0.066	0.084	0.026
-20	0.031	0.049	-0.049	-0.004			5	0.025	0.018	0.046	0.019
-19	-0.006	0.102	-0.058	0.005			6	0.021	0.014	0.025	0.025
-18	0.017	0.086	-0.039	-0.001			7	0.033	0.026	0.008	0.036
-17	0.064	0.087	0.011	0.031			8	0.039	0.032	0.009	0.030
-16	0.041	0.098	0.013	0.038			9	0.037	0.030	0.023	0.026
-15	0.030	0.124	0.006	0.023			10	0.022	0.015	-0.007	0.032
-14	0.046	0.106	0.005	0.027			11	0.062	0.054	0.001	0.011
-13	0.035	0.124	0.381	0.007			12	0.088	0.079	0.021	0.050
-12	0.017	0.066	0.483	0.010			13	0.034	0.027	0.049	0.019
-11	0.040	0.172	0.248	0.003			14	0.008	0.002	0.039	0.033
-10	0.120	0.079	0.098	-0.005			15	0.00	-0.009	0.029	0.048
-9	0.275	0.139	0.045	0.009			16	-0.01	-0.017	0.012	0.045
-8	0.402	0.168	0.090	0.005			17	0.034	0.027	0.015	0.035
-7	0.505	0.106	0.088	0.014			18	0.032	0.025	0.021	0.032
-6	0.271	0.121	0.090	0.013			19	0.051	0.043	0.054	0.017
-5	0.336	0.193	0.070	0.016			20	0.065	0.057	0.010	0.018
-4	0.315	0.257	0.059	0.019			21	0.245	0.231	0.043	0.017
-3	0.153	0.336	0.093	0.033			22	0.580	0.554	0.039	0.034
-2	0.445	0.371	0.054	0.028			23	0.344	0.327	0.101	0.050
-1	0.482	0.355	0.041	0.024			24	0.240	0.226	0.128	0.053
0	0.388	0.350	0.046	0.023	0.011	0.021	25	0.196	0.184	0.243	0.018
1	0.408	0.301	0.035	0.020	0.029	0.019	26	0.130	0.120	0.568	0.033
2	0.527	0.275	0.049	0.018	0.054	0.025	27	0.419	0.399	0.432	0.038
3	0.487	0.411	0.080	0.026	0.051	0.059	28	0.335	0.318	0.474	0.044
4	0.768	0.415	0.170	0.030	0.057	0.046	29	0.357	0.339	0.208	0.044
5	0.647	0.309	0.216	0.039	0.115	0.105	30	0.298	0.282	0.486	0.041
6	0.787	0.133	0.149	0.033	0.143	0.093	31	0.380	0.361	0.473	0.026
7	0.677	0.104	0.224		0.098	0.088	32	0.600	0.574	0.657	0.030
8	0.229	0.108	0.286		0.119	0.097	33	0.434	0.413	0.620	0.029
9	0.247	0.098	0.373		0.083	0.094					
10	0.247	0.212	0.445		0.156	0.123					
11	0.486	0.120	0.380		0.116	0.097					
12	0.441	0.097	0.239			0.092					

Appendix Table 4

Titration for total Igand isotypes showing mean OD values obtained in *F. hepatica* and *F. gigantica* infected and uninfected sheep using Fh-cathepsin. as antigen

Appendix Table 4.75

Titration for total Ig showing the mean OD (450 nm)values obtained in *F. hepatica* and *F. gigantica* infected and uninfected sheep.

Cathepsin	B	N	P1	P2	Cathepsin	B	N	P1	P2
4	0.254	0.629	1.137	1.215	4	0.142	0.518	1.187	1.141
2	0.248	0.489	0.968	1.069	2	0.149	0.337	0.337	1.124
1	0.146	0.333	1.192	1.278	1	0.102	0.391	1.169	1.269
Serum	B	N	P1	P2	Serum	B	N	P1	P2
50	0.025	0.911	1.663	1.602	50	0.015	0.626	1.416	1.483
100	0.015	0.607	1.541	1.471	100	0.015	0.342	1.369	1.355
200	0.011	0.571	1.537	1.391	200	0.015	0.227	1.270	1.262
400	0.01	0.433	1.468	1.269	400	0.014	0.200	1.175	1.050
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.029	0.523	1.563	1.583	1000	0.015	0.424	0.102	1.459
2000	0.021	0.315	1.541	1.471	2000	0.015	0.227	1.270	1.262
4000	-0.032	0.254	1.552	1.466	4000	-0.04	0.185	1.150	1.150
8000	0.008	0.156	1.251	1.190	8000	0.006	0.056	0.797	1.262

Appendix Table 4.76

Titration for IgG_1 showing the mean OD (450nm) values obtained in *F. hepatica* and *F. gigantica* infected and uninfected sheep

Antigen (µg/ml) Titration IgG_1

Cathepsin	B	N	P1	P2	Cathepsin	B	N	P1	P2
4	0.031	0.439	1.917	1.204	4	0.041	0.059	0.365	1.692
2	0.055	0.361	1.422	1.003	2	0.039	0.040	0.280	1.107
1	0.043	0.265	0.979	0.750	1	0.027	0.031	0.245	0.543

Serum	B	N	P1	P2	Serum	B	N	P1	P2
50	0.051	0.090	0.691	0.502	50	0.050	0.064	0.179	0.368
100	0.049	0.040	0.602	0.419	100	0.043	0.051	0.165	0.317
200	0.065	0.087	0.230	0.282	200	0.023	0.034	0.160	0.259
400	0.070	0.060	0.135	0.171	400	0.020	0.029	0.068	0.090
McAb	B	N	P1	P2	McAb	B	N	P1	P2
20	0.049	0.186	0.602	0.419	20	0.043	0.051	0.165	0.317
40	0.068	0.194	0.480	0.371	40	0.060	0.057	0.135	0.168
80	0.081	0.136	0.211	0.224	80	0.071	0.075	0.110	0.149
160	0.087	0.102	0.151	0.158	160	0.074	0.071	0.094	0.117
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.055	0.140	1.619	0.278	1000	0.084	0.114	0.265	0.346
2000	0.044	0.110	1.216	0.222	2000	0.066	0.085	0.187	0.210
4000	0.041	0.062	0.849	0.129	4000	0.059	0.065	0.112	0.122
8000	0.039	0.050	0.490	0.087	8000	0.052	0.052	0.088	0.092
16000	0.045	0.038	0.287	0.067	16000	0.054	0.052	0.070	0.068
32000	0.048	0.043	0.153	0.059	32000	0.060	0.054	0.063	0.068

Appendix Table 4.77

Titrations for total IgM showing mean OD (450 nm) values obtained *in F. hepatica* and *F. gigantica* infected and uninfected sheep Fh-Cathepsin (µg/ml) as antigen

Antigen (µg/ml) Titration									
Cathepsin	B	N	P1	P2	Cathepsin	B	N	P1	P2
4	0.190	1.406	0.517	1.327	4	0.230	1.114	1.483	1.946
2	0.245	1.428	1.151	1.380	2	0.234	1.153	1.152	1.326
1	0.049	0.670	0.414	0.452	1	0.074	0.362	0.503	0.820

Serum	B	N	P1	P2	Serum	B	N	P1	P2
50	0.049	0.067	0.414	0.452	50	0.027	0.268	0.426	0.350
100	0.055	0.498	0.255	0.367	100	0.250	0.170	0.523	0.266
200	0.044	0.333	0.182	0.311	200	0.032	0.105	0.248	0.192
400	0.047	0.172	0.121	0.202	400	0.033	0.119	0.199	0.089

Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.100	0.075	1.150	0.965	1000	0.060	0.343	0.646	0.560
2000	0.035	0.389	0.681	0.570	2000	0.025	0.170	0.523	0.266
4000	0.028	0.162	0.251	0.127	4000	0.014	0.073	0.168	0.129
8000	0.032	0.101	0.189	0.154	8000	0.019	0.027	0.073	0.046

Appendix Table 4.78

Titrations for IgG_2 showing the mean OD (450 nm) values obtained in *F. hepatica* and *F. gigantica* infected and uninfected sheep Fh-Cathepsin (µg/ml) as antigen

Cathepsin	B	N	P1	P2	Cathepsin	B	N	P1	P2
4	0.052	0.084	0.195	0.088	4	0.042	0.050	0.050	0.040
2	0.048	0.067	0.138	0.073	2	0.041	0.059	0.052	0.050
1	0.051	0.073	0.086	0.072	1	0.051	0.057	0.059	0.044

Serum	B	N	P1	P2	Serum	B	N	P1	P2
50	0.123	0.229	0.203	0.149	50	0.100	0.129	0.129	0.114
100	0.054	0.075	0.088	0.074	100	0.051	0.057	0.059	0.044

McAb	B	N	P1	P2	McAb	B	N	P1	P2
10	0.108	0.285	0.250	0.165	10	0.097	0.152	0.131	0.124
20	0.123	0.229	0.203	0.149	20	0.100	0.129	0.129	0.114
40	0.102	0.102	0.138	0.122	40	0.084	0.137	0.103	0.106
80	0.089	0.103	0.124	0.122	80	0.079	0.09	0.09	0.091

Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.082	0.123	0.125	0.183	1000	0.084	0.019	0.112	0.117
2000	0.066	0.088	0.094	0.112	2000	0.070	0.074	0.076	0.079
4000	0.057	0.079	0.076	0.078	4000	0.057	0.065	0.063	0.064
8000	0.056	0.072	0.061	0.062	8000	0.058	0.054	0.052	0.055
16000	0.055	0.062	0.050	0.060	16000	0.051	0.048	0.053	0.052
32000	0.050	0.064	0.059	0.068	32000	0.063	0.051	0.058	0.060

Appendix Table 4.79

Titrations for IgA showing the mean OD (450 nm) values obtained in *F. hepatica* and *F. gigantica* infected and uninfected sheep

F. hepatica

Cathepsin	B	N	P1	P2
4	0.041	0.391	0.565	0.811
2	0.193	0.369	0.491	0.763
1	0.078	0.339	0.405	0.607

F.gigantica

Cathepsin	B	N	P1	P2
4	0.044	0.920	0.751	0.591
2	0.051	0.778	0.776	0.556
1	0.061	0.701	0.614	0.495

Serum	B	N	P1	P2
50	0.082	0.102	0.078	0.119
100	0.053	0.082	0.066	0.083

Serum	B	N	P1	P2
50	0.075	0.084	0.076	0.087
100	0.074	0.064	0.062	0.075

McAb	B	N	P1	P2
10	0.079	0.100	0.067	0.079
20	0.082	0.102	0.078	0.083
40	0.079	0.099	0.075	0.087
80	0.075	0.095	0.074	0.082

McAb	B	N	P1	P2
10	0.070	0.071	0.072	0.072
20	0.075	0.084	0.076	0.080
40	0.072	0.093	0.080	0.076
80	0.069	0.074	0.071	0.072

Conj.	B	N	P1	P2
1000	0.053	0.082	0.066	0.119
2000	0.039	0.050	0.042	0.068
4000	0.029	0.031	0.025	0.041
8000	0.028	0.026	0.018	0.030
16000	0.022	0.025	0.017	0.027
32000	0.026	0.027	0.018	0.026

Conj.	B	N	P1	P2
1000	0.074	0.064	0.062	0.087
2000	0.037	0.040	0.041	0.050
4000	0.033	0.029	0.029	0.037
8000	0.024	0.023	0.029	0.028
16000	0.023	0.021	0.019	0.021
32000	0.031	0.022	0.027	0.029

Table 4.80 Titration for total IgG showing the mean OD values obtained in *F. hepatica* and *F. gigantica* infected and uninfected Cattle, Fh-Cathepsin (µg/ml) as antigen

F. hepatica

Cathepsin	B	N	P1	P2
4	0.013	0.047	1.243	1.017
2	0.013	0.038	1.289	0.924
1	0.012	0.022	1.134	0.762

F. gigantica

Cathepsin	B	N	P1	P2
4	0.017	0.176	1.227	1.460
2	0.015	0.141	1.250	1.379
1	0.008	0.077	1.201	1.370

F. hepatica

Serum	B	N	P1	P2
50	0.001	0.038	1.460	1.144
100	-0.001	0.021	1.276	0.917
200	-0.001	0.015	1.063	0.669
400	-0.002	0.011	0.816	0.413
800	0.005	0.006	0.534	0.264
1600	0.000	0.012	0.390	0.175

F. gigantica

Serum	B	N	P1	P2
50	0.006	0.167	1.494	1.57
100	0.005	0.094	1.309	1.449
200	0.002	0.05	1.131	1.33
400	0.003	0.032	0.824	1.211
800	0.007	0.01	0.607	0.754
1600	0.003	0.01	0.292	0.661

F. hepatica

conj.	B	N	P1	P2
1000	0.034	0.105	1.332	1.086
2000	0.022	0.073	1.275	0.847
4000	0.011	0.05	1.053	0.637
8000	0.011	0.032	0.819	0.474
16000	0.005	0.029	0.57	0.28
32000	0.008	0.014	0.351	0.167

F. gigantica

conj.	B	N	P1	P2
1000	0.04	0.197	1.31	1.472
2000	0.022	0.108	1.192	1.272
4000	0.014	0.084	1.065	1.15
8000	0.012	0.06	0.812	0.952
16000	0.011	0.03	0.585	0.969
32000	0.015	0.021	0.355	0.415

Table 4.81:_Titration for IgG1 showing the mean OD values obtained in *F. hepatica* and *F. gigantica* infected and uninfected Cattle, Fh-Cathepsin (µg/ml) as antigen

F. hepatica					*F. gigantica*				
Cathepsin	B	N	P1	P2	Cathepsin	B	N	P1	P2
4	0.017	0.100	1.479	1.361	4	0.034	0.393	1.534	1.702
2	0.021	0.077	1.599	1.297	2	0.019	0.363	1.606	1.542
1	0.020	0.057	1.490	1.224	1	0.017	0.177	1.476	1.684
F. hepatica					*F. gigantica*				
Serum	B	N	P1	P2	Serum	B	N	P1	P2
50	0.014	0.119	1.609	1.461	50	0.011	0.407	1.628	1.766
100	0.01	0.094	1.411	1.378	100	0.012	0.306	1.608	1.742
200	0.014	0.048	1.497	1.213	200	0.022	0.124	1.524	1.676
400	0.017	0.032	1.347	0.911	400	0.013	0.08	1.331	1.522
800	0.009	0.023	1.119	0.666	800	0.012	0.045	1.099	1.444
1600	0.013	0.023	0.872	0.483	1600	0.014	0.039	0.78	1.105
F. hepatica					*F. gigantica*				
conj.	B	N	P1	P2	conj.	B	N	P1	P2
1000	0.046	0.092	1.897	1.230	1000	0.050	0.250	1.934	1.859
2000	0.026	0.056	1.669	0.894	2000	0.026	0.137	1.458	1.806
4000	0.022	0.040	1.288	0.650	4000	0.024	0.081	1.041	1.389
8000	0.013	0.017	0.851	0.348	8000	0.013	0.039	0.618	0.942
16000	0.011	0.015	0.542	0.193	16000	0.011	0.024	0.428	0.594
32000	0.010	0.011	0.394	0.104	32000	0.010	0.022	0.312	0.401

Table:4.82 Titration for IgM showing the mean OD values obtained in *F. hepatica* and *F. gigantica* infected and uninfected Cattle, Fh-Cathepsin (µg/ml) as antigen

F. hepatica					*F. gigantica*				
Antigen conc.	B	N	P1	P2	Antigen conc.	B	N	P1	P2
4	0.013	0.256	0.607	0.620	4	0.007	0.758	0.722	1.185
2	0.005	0.204	0.532	0.502	2	0.004	0.664	0.562	1.008
1	0.008	0.162	0.354	0.439	1	0.012	0.563	0.484	0.940
F. hepatica					*F. gigantica*				
Serum	B	N	P1	P2	Serum	B	N	P1	P2
100	0.069	0.198	0.741	0.512	100	0.042	0.578	0.912	0.857
200	0.049	0.109	0.562	0.457	200	0.035	0.511	0.803	0.749
400	0.035	0.099	0.279	0.31	400	0.041	0.365	0.451	0.384
800	0.022	0.072	0.221	0.124	800	0.025	0.219	0.354	0.325
1600	0.012	0.032	128	0.081	1600	0.033	0.102	0.234	0.241
3200	0.003	0.021	0.087	0.033	3200	0.054	0.088	0.142	0.149
F. hepatica					*F. gigantica*				
conj.	B	N	P1	P2	conj.	B	N	P1	P2
1000	0.029	0.287	0.807	0.605	1000	0.027	0.627	0.927	0.957
2000	0.013	0.176	0.477	0.367	2000	0.022	0.596	0.709	0.900
4000	0.011	0.103	0.358	0.205	4000	0.021	0.355	0.473	0.424
8000	0.009	0.067	0.199	0.154	8000	0.015	0.234	0.346	0.313
16000	0.06	0.037	0.108	0.063	16000	0.010	0.126	0.216	0.213
32000	0.003	0.028	0.062	0.046	32000	0.013	0.067	0.172	0.177

Table 4.83 Titration for IgG2 showing the mean OD values obtained in *F. hepatica* and *F. gigantica* infected and uninfected Cattle, Fh-Cathepsin (µg/ml) as antigen

F. hepatica					*F. gigantica*				
Cathepsin	B	N	P1	P2	Cathepsin	B	N	P1	P2
4	0.078	0.1	0.139	0.591	4	0.078	0.232	0.257	0.436
2	0.054	0.097	0.1	0.36	2	0.054	0.132	0.174	0.332
1	0.076	0.113	0.116	0.272	1	0.084	0.084	0.136	0.246
F. hepatica					*F. gigantica*				
Serum	B	N	P1	P2	Serum	B	N	P1	P2
50	0.051	0.074	0.137	0.658	50	0.044	0.112	0.285	0.735
100	0.03	0.035	0.055	0.171	100	0.031	0.042	0.104	0.208
200	0.018	0.018	0.032	0.126	200	0.018	0.032	0.048	0.071
400	0.019	0.008	0.021	0.061	400	0.018	0.028	0.029	0.038
800	0.014	0.009	0.016	0.039	800	0.010	0.010	0.018	0.018
1600	0.005	0.006	0.01	0.017	1600	0.009	0.005	0.014	0.011
F. hepatica					*F. gigantica*				
conj.	B	N	P1	P2	conj.	B	N	P1	P2
1000	0.084	0.171	0.330	1.034	1000	0.114	0.308	0.442	0.493
2000	0.041	0.088	0.194	0.709	2000	0.073	0.160	0.277	0.314
4000	0.035	0.057	0.114	0.330	4000	0.045	0.010	0.135	0.141
8000	0.025	0.032	0.062	0.191	8000	0.030	0.048	0.075	0.160
16000	0.024	0.021	0.340	0.103	16000	0.023	0.028	0.046	0.051
32000	0.015	0.019	0.270	0.061	32000	0.035	0.023	0.046	0.035

Table 4.84 Titration for IgA showing the mean OD values obtained in *F. hepatica* and *F. gigantica* infected and uninfected Cattle, Fh-Cathepsin (µg/ml) as antigen

F. hepatica						*F. gigantica*			
Cathepsin	B	N	P1	P2	Cathepsin	B	N	P1	P2
4	0.037	0.160	0.214	0.180	4	0.070	0.108	0.325	0.151
2	0.028	0.037	0.303	0.100	2	0.028	0.037	0.290	0.111
1	0.032	0.023	0.255	0.058	1	0.009	0.023	0.255	0.106
F. hepatica						*F. gigantica*			
Serum	B	N	P1	P2	Serum	B	N	P1	P2
50	0.034	0.044	0.385	0.109	50	0.034	0.044	0.325	0.193
100	0.029	0.025	0.203	0.156	100	0.029	0.035	0.187	0.156
200	0.030	0.022	0.167	0.070	200	0.024	0.026	0.081	0.119
400	0.024	0.028	0.088	0.024	400	0.019	0.017	0.041	0.089
800	0.011	0.015	0.024	0.014	800	0.014	0.008	0.024	0.065
1600	0.011	0.020	0.019	0.021	1600	0.009	0.001	0.029	0.054
F. hepatica						*F. gigantica*			
conj.	B	N	P1	P2	conj.	B	N	P1	P2
1000	0.034	0.044	0.185	0.090	1000	0.037	0.060	0.160	0.190
2000	0.029	0.025	0.103	0.056	2000	0.028	0.037	0.103	0.100
4000	0.030	0.040	0.067	0.035	4000	0.032	0.023	0.055	0.058
8000	0.024	0.028	0.038	0.024	8000	0.038	0.023	0.039	0.037
16000	0.011	0.015	0.024	0.014	16000	0.026	0.012	0.020	0.030
32000	0.011	0.020	0.019	0.021	32000	0.027	0.010	0.180	0.018

APPENDIX 4

Adjusted OD values for antibody response to *F. hepatica* cathepsin L1 protease

Experiment One (1): Adjusted values for *F. hepatica* (British and Peruvian strain) infected sheep and uninfected sheep

Appendix Table 4.85

Adjusted total Ig OD values

WPI	SH.5	SH. 6	SH. 7	SH. 9	SH. 10	SH. 8
-2	0.004	0.033	0.033	0.028	0.210	0.040
-1	-0.022	0.001	0.081	-0.095	0.063	0.074
0	-0.015	-0.004	0.147	-0.043	0.126	0.095
1	0.019	-0.033	0.101	-0.088	0.072	0.088
2	0.013	0.930	0.419	0.365	0.615	0.035
3	-0.008	0.973	1.227	0.559	1.304	0.056
4	0.297	1.166	1.158	0.840	1.811	0.038
5	0.512	1.329	1.147	0.608	1.392	0.026
6	1.149	1.150	1.199	0.668	1.500	0.093
7	1.246	1.061	1.188	0.889	1.898	0.021
8	1.163	1.347	1.080	0.709	1.574	0.067
9	1.238	1.298	1.102	0.679	1.520	0.078
10	0.839	1.272	1.077	0.593	1.365	0.083
11	1.267	1.397	1.147	0.608	1.392	0.065
12	1.111	1.416	1.227	0.866	1.858	0.110
13	1.061	1.290	0.958	0.694	1.547	0.057
14	1.466	0.786	0.435	0.851	1.831	0.088
15		1.244	0.699	0.600	1.379	0.043
16		0.918	1.037	0.686	1.534	0.078
5.5		0.893	1.216	0.413	1.041	0.083

Adjusted IgG_1 OD values

WPI	SH.5	SH. 6	SH. 7	SH. 9	SH.10	SH. 8
-2	0.004	0.007	0.013	0.012	0.023	0.020
-1	0.001	0.006	0.021	-0.014	0.034	0.024
0	0.002	0.010	-0.002	0.027	0.019	0.021
1	0.026	0.012	-0.001	-0.017	0.048	0.021
2	0.007	0.376	0.312	0.048	0.068	0.019
3	0.192	1.004	0.864	0.169	0.047	0.018
4	0.181	1.014	0.873	0.253	0.314	0.019
5	0.264	1.198	1.035	0.241	0.200	0.017
6	0.531	1.324	1.146	0.209	0.284	0.020
7	0.717	1.038	0.894	0.243	0.304	0.026
8	0.545	1.156	0.848	0.270	0.265	0.025
9	0.334	1.034	0.891	0.348	0.405	0.020
10	0.337	1.027	0.884	0.237	0.232	0.026
11	0.446	1.239	0.895	0.173	0.270	0.026
12	0.404	0.816	0.699	0.245	0.169	0.020
13	0.958	1.026	0.884	0.270	0.205	0.029
14		0.790	0.676	0.403	0.100	0.021
15		0.943	0.634	0.424	0.128	0.025
16		0.328	0.269	0.288	0.084	0.028
17		0.470	0.394	0.218	0.063	0.024

Appendix Table 4.86

Adjusted IgM OD values

WPI	SH.5	SH. 6	SH. 7	SH. 9	SH.10	SH. 8
-2	0.017	0.037	0.036	0.036	0.014	0.035
-1	0.027	0.023	0.019	0.117	0.069	0.071
0	0.045	0.034	0.032	0.116	0.092	0.011
1	0.079	0.046	0.048	0.150	0.097	0.047
2	0.070	0.065	0.191	0.328	0.159	0.041
3	0.109	0.066	0.318	0.329	0.157	0.041
4	0.138	0.081	0.090	0.384	0.177	0.039
5	0.153	0.196	0.230	0.322	0.139	0.014
6	0.149	0.192	0.226	0.255	0.143	-0.006
7	0.166	0.423	0.506	0.270	0.095	0.039
8	0.098	0.483	0.578	0.323	0.159	0.033
9	0.098	0.313	0.372	0.230	0.204	0.039
10	0.090	0.267	0.571	0.161	0.123	0.020
11	0.064	0.313	0.372	0.226	0.164	0.033
12	0.090	0.194	0.228	0.266	0.140	0.020
13	0.140	0.271	0.322	0.228	0.234	0.043
14		0.495	0.594	0.275	0.101	0.044
15		0.359	0.428	0.222	0.152	0.020
16		0.158	0.184	0.237	0.091	0.030
17		0.127	0.062	0.216	0.054	0.053

Adjusted IgG_2 OD values

WPI	SH.5	SH. 6	SH. 7	SH. 9	SH.10	SH. 8
-2	0.021	0.035	0.024	0.012	0.031	0.021
-1	0.038	0.055	0.012	0.014	0.018	0.018
0	0.023	0.037	0.025	0.008	0.018	0.031
1	0.038	0.060	0.041	0.027	0.034	0.028
2	0.023	0.029	0.033	0.046	0.043	0.024
3	0.046	0.021	0.017	0.039	0.015	0.013
4	0.034	0.027	0.027	0.052	0.039	0.020
5	0.055	0.060	0.020	0.055	0.048	0.008
6	0.036	0.042	0.040	0.056	0.045	0.005
7	0.028	0.040	0.039	0.013	0.053	0.014
8	0.050	0.053	0.007	0.017	0.066	0.048
9	0.059	0.027	0.007	0.040	0.101	0.042
10	0.030	0.024	0.043	0.060	0.078	0.039
11	0.023	0.056	0.039	0.037	0.080	0.041
12		0.050	0.043	0.040	0.054	0.015
13		0.058	0.021	0.005	0.050	0.011
14		0.024	0.010	0.052	0.033	0.011
15		0.031	0.041	0.073	0.052	0.013
16		0.053	0.043	0.059	0.040	0.000
17		0.053	0.007	0.053	0.045	0.005

Appendix Table 4.87

Adjusted IgA values

WPI	SH.5	SH. 6	SH. 7	SH. 9	SH.10	SH. 8
-2	0.017	0.021	0.026	0.018	0.024	0.021
-1	0.022	0.030	0.008	0.011	0.020	0.018
0	0.026	0.037	0.018	0.019	0.018	0.018
1	0.027	0.027	0.035	0.007	0.023	0.015
2	0.049	0.034	0.037	0.021	0.031	0.014
3	0.023	0.046	0.012	0.011	0.011	0.012
4	0.022	0.021	0.009	0.016	0.029	0.004
5	0.030	0.027	0.008	0.021	0.017	0.016
6	0.023	0.049	0.012	0.023	0.008	0.022
7	0.032	0.042	0.020	0.024	0.010	0.018
8	0.022	0.034	0.020	0.029	0.012	0.006
9	0.033	0.035	0.014	0.042	0.041	0.025
10	0.031	0.034	0.020	0.029	0.034	0.013
11	0.024	0.031	0.035	0.029	0.028	0.023
12	0.030	0.069	0.034	0.042	0.045	0.029
13	0.032	0.075	0.035	0.039	0.029	0.017
14		0.049	0.024	0.084	0.030	0.022
15		0.024	0.033	0.056	0.036	0.012
16		0.022	0.020	0.043	0.046	0.021
17		0.031	0.014	0.037	0.029	0.011

Experiment two (2): Adjusted values for *F. hepatica* (British strain) infected and uninfected sheep

Appendix Table 4.88

Adjusted IgG OD values

WPI	sh. 24	sh.26	sh. 28	sh.30	sh. 22	sh. 32
-2	0.064	0.050	0.048	-0.048	0.039	0.046
0	0.022	0.154	0.072	0.090	0.039	-0.004
2	0.360	0.432	0.295	0.137	0.053	0.008
4	0.417	0.268	0.072	0.295	0.026	0.004
6	0.404	0.696	0.241	0.317	0.075	-0.026
8	1.230	1.154	0.980	1.079	0.058	-0.014
9	0.806	0.984	0.966	1.015	0.094	0.051
10	1.168	1.066	1.055	1.004	0.104	-0.030
11	1.236	1.019	1.066	1.053	0.048	-0.085
12	1.137	1.148	1.034	1.042	-0.028	-0.033
13	1.172	1.013	1.017	0.941	0.041	-0.046
14	1.083	1.004	0.946	0.961	0.009	-0.002
15	1.080	1.088	0.996	0.938	0.017	-0.088
16	1.091	0.988	0.860	1.004	0.041	-0.049
17	1.080	0.973	0.902	1.079	0.026	0.016
18	0.998	1.038	0.905	0.826	-0.056	-0.061
19	1.043	1.027	0.768	0.332	0.072	-0.049
20	0.922	1.024	0.905	0.581	0.074	-0.048
21	1.196	0.779	0.892	0.900	-0.056	-0.043
22			0.899	1.069	-0.052	0.002

Adjusted IgG_1 OD values

WPI	sh.24	sh.26	sh.28	sh.30	sh.22	sh.32
-2	0.009	0.034	-0.029	-0.029	0.010	-0.001
0	0.071	0.019	-0.009	-0.024	0.023	0.006
2	0.089	0.048	-0.017	-0.024	0.023	-0.003
4	0.073	0.035	-0.002	-0.024	0.015	-0.003
6	0.159	0.181	0.023	-0.002	0.037	0.012
8	1.028	0.943	-0.042	0.081	-0.001	0.004
9	1.029	1.107	0.000	0.517	0.012	-0.003
10	0.911	1.109	1.085	0.606	0.043	-0.003
11	1.051	1.284	1.303	0.745	0.017	-0.008
12	1.193	1.332	1.330	1.291	0.010	-0.012
13	1.324	1.466	1.467	1.059	0.010	-0.012
14	1.258	1.174	1.384	0.999	-0.001	0.001
15	1.211	1.008	1.457	1.309	0.006	-0.005
16	1.237	1.109	1.552	1.299	0.006	-0.010
17	1.172	1.087	1.474	0.997	0.008	-0 010
18	1.290	1.321	1.401	0.715	0.012	-0.008
19	1.027	1.113	1.399	0.532	0.030	-0.012
20	0.510	0.870	1.348	0.434	0.008	-0.012
21	0.262	0.376	0.508	0.219	0.021	-0.012
22	0.189	0.093	0.067	0.943	0.037	0.004

Appendix Table 4.89

Adjusted IgM OD values

WPI	sh. 24	sh.26	sh. 28	sh.30	sh. 22	sh. 32
-2	0.523	0.243	0.221	0.339	0.576	-0.049
0	0.389	0.182	0.363	0.377	0.653	0.073
2	0.374	0.196	0.417	0.466	0.392	-0.128
4	0.378	0.327	0.421	0.353	0.387	-0.007
6	0.723	0.356	0.393	0.440	0.582	-0.028
8	0.405	0.374	0.357	0.368	0.561	-0.028
9	0.865	0.239	0.257	0.396	0.478	-0.034
10	0.401	0.426	0.262	0.288	0.449	-0.117
11	0.637	0.392	0.342	0.372	0.372	-0.185
12	0.665	0.245	0.303	0.373	0.191	-0.034
13	0.407	0.035	0.337	0.322	0.582	-0.054
14	0.421	0.082	0.315	0.191	0.526	-0.034
15	0.708	0.279	0.373	0.252	0.446	-0.099
16	0.456	0.206	0.464	0.297	0.271	-0.054
17	0.676	0.185	0.468	0.463	0.253	-0.096
18	1.192	0.153	0.503	0.368	0.295	-0.022
19	0.055	0.047	0.441	0.423	0.434	0.159
20	1.443	0.070	0.409	0.311	0.259	0.111
21	0.645	0.151	0.461	0.238	0.244	-0.063
22	0.606	0.165	0.431	0.487	0.558	0.014

Adjusted IgG_2 OD values

WPI	sh. 24	sh.26	sh. 28	sh.30	sh. 22	sh. 32
-2	-0.012	0.024	0.001	-0.001	-0.003	-0.004
0	0.021	0.047	0.007	0.000	0.010	-0.003
2	0.019	0.046	0.009	0.001	0.009	-0.004
4	-0.013	0.023	0.010	0.000	0.002	-0.003
6	0.004	0.035	0.009	0.003	-0.001	-0.003
8	0.064	0.077	0.008	0.021	0.004	-0.005
9	-0.046	0.000	0.011	0.027	-0.004	0.001
10	0.010	0.039	0.012	0.026	-0.005	-0.007
11	-0.015	0.021	0.010	0.019	0.004	-0.011
12	-0.017	0.020	0.015	0.014	-0.003	-0.004
13	-0.031	0.010	0.008	0.014	-0.006	-0.005
14	0.001	0.033	0.008	0.010	-0.004	-0.001
15	-0.004	0.029	0.005	0.009	-0.001	-0.002
16	-0.026	0.014	0.009	0.007	-0.009	-0.007
17	-0.032	0.010	0.010	0.015	0.003	-0.008
18	-0.032	0.010	0.005	0.015	0.009	0.002
19	-0.031	0.010	0.004	0.008	-0.010	0.000
20	-0.037	0.006	-0.001	0.005	0.010	-0.006
21	-0.024	0.015	0.004	0.000	-0.003	-0.006
22					-0.014	-0.005

Appendix Table 4.90

Adjusted IgA values

WPI	sh. 24	sh.26	sh. 28	sh.30	sh. 22	sh. 32
-2	0.003	0.012	0.031	0.010	0.038	-0.019
0	0.012	0.032	0.011	0.015	0.010	-0.043
2	0.021	0.092	0.022	0.024	-0.005	-0.014
4	0.023	0.115	0.041	0.019	0.024	-0.034
6	0.072	0.108	0.044	0.017	-0.010	-0.029
8	0.014	0.135	0.016	0.075	0.010	-0.014
9	0.001	0.101	0.012	0.069	-0.005	-0.010
10	0.030	0.258	0.011	0.066	0.005	-0.043
11	0.014	0.075	0.016	0.073	-0.048	-0.034
12	0.034	0.193	0.025	0.075	-0.043	-0.019
13	0.012	0.041	0.025	0.062	-0.005	0.034
14	0.037	0.043	0.018	0.041	-0.010	0.010
15	0.032	0.041	0.024	0.042	-0.024	0.029
16	0.017	0.034	0.041	0.038	-0.019	-0.014
17	0.030	0.119	0.040	0.034	0.058	0.096
18	0.034	0.066	0.041	0.031	0.029	0.024
19	0.034	0.075	0.029	0.034	0.000	0.058
20	0.059	0.019	0.039	0.036	-0.019	0.053
21	0.039	0.017	0.047	0.024	-0.019	0.053
22			0.033	0.038	-0.062	0.043

Experiment three (3): Adjusted values for *F. gigantica* (Kenyan strain) infected and uninfected sheep

Appendix Table 4.91

Adjusted total Ig OD values

	Infected								Uninfected						
WPI	SH.11	SH.12	SH. 13	SH. 14	SH. 15	Mean (+)		SEM	SH.16	SH. 17	SH. 18	SH. 19	Mean (-	StDev	SEM
-2	-0.128	-0.004	-0.053	0.067	-0.021	-0.028	0.064	0.032	-0.077	-0.015	-0.011	-0.024	-0.003	0.027	0.009
0	-0.029	0.000	-0.041	0.148	-0.038	0.008	0.071	0.036	-0.025	0.008	0.013	-0.009	-0.023	0.015	0.016
2	0.564	0.120	0.057	0.251	0.009	0.200	0.199	0.100	-0.008	0.015	-0.049	-0.051	-0.028	0.028	0.010
4	0.317	0.249	0.222	0.312	0.196	0.259	0.048	0.024	-0.010	-0.025	-0.021	-0.057	-0.039	0.017	0.017
6	0.417	0.189	0.746	0.354	0.658	0.473	0.203	0.102	-0.015	-0.012	-0.085	-0.045	-0.045	0.029	0.020
8	0.344	0.291	0.705	0.360	0.828	0.506	0.218	0.109	-0.003	-0.025	-0.096	-0.054	-0.014	0.035	0.026
9	0.582	0.321	0.605	0.754	1.209	0.694	0.293	0.146	0.013	0.016	0.007	-0.090	0.000	0.044	0.019
10	0.270	0.682	0.743	0.858	0.749	0.660	0.203	0.102	0.025	-0.052	0.034	-0.006	-0.020	0.034	0.027
11	0.539	0.821	0.858	0.823	0.635	0.735	0.125	0.063	0.037	-0.077	0.015	-0.054	-0.063	0.047	0.026
12	0.660	0.731	0.822	0.779	0.428	0.684	0.139	0.069	0.006	-0.122	-0.066	-0.070	-0.054	0.046	0.026
13	0.983	0.631	0.435	0.697	0.395	0.628	0.211	0.105	0.020	-0.100	-0.081	-0.054	-0.037	0.046	0.025
14	0.548	0.816	0.476	0.823	0.188	0.570	0.237	0.118	-0.012	-0.066	0.020	-0.090	-0.026	0.043	0.021
15	0.417	0.683	0.927	0.541	0.120	0.538	0.269	0.134	-0.018	0.029	-0.067	-0.048	-0.038	0.036	0.007
16	0.344	0.582		0.553	0.618	0.524	0.107	0.062	-0.022	-0.039	-0.036	-0.054	-0.012	0.011	0.009
17	0.582	0.474		0.597	0.441	0.524	0.067	0.039	-0.022	-0.030	0.012	-0.006	-0.017	0.016	0.029
18	0.134	0.562		0.390	0.386	0.368	0.153	0.088	0.025	-0.025	0.028	-0.095	-0.029	0.050	0.021
19	0.033	0.541		0.411	0.296	0.320	0.187	0.108	-0.012	-0.001	-0.011	-0.090	-0.013	0.036	0.024
20	0.044	0.472		0.492	0.328	0.334	0.179	0.103	-0.018	0.030	0.013	-0.077	-0.033	0.041	0.008
21	0.149	0.398		0.456	0.354	0.339	0.116	0.067	-0.022	-0.019	-0.037	-0.055	-0.037	0.014	0.029
22	0.018	0.375		0.586	0.315	0.324	0.203	0.117	0.037	-0.030	-0.105	-0.048	-0.029	0.051	0.021

Appendix Table 4.92

Adjusted IgG_1 OD values

	Infected								Uninfected						
WPI	SH.11	SH.12	SH. 13	SH. 14	SH. 15	Mean (+)	StDev	SEMean	SH.16	SH. 17	SH. 18	SH. 19	TrMean	StDev	SEMean
-2	0.059	0.046	0.021	0.025	0.026	0.035	0.016	0.007	0.031	0.036	0.045	0.020	0.03	0.01	0.01
0	-0.045	-0.058	0.008	0.010	0.009	-0.015	0.034	0.015	0.037	0.042	0.051	0.026	0.04	0.01	0.01
2	-0.035	-0.048	0.011	0.006	0.004	-0.012	0.027	0.012	0.037	0.042	0.051	0.026	0.04	0.01	0.01
4	0.016	0.003	0.020	0.072	0.080	0.038	0.035	0.016	0.033	0.038	0.047	0.022	0.04	0.01	0.01
6	0.149	0.136	0.006	0.165	0.186	0.128	0.071	0.032	0.043	0.048	0.057	0.032	0.05	0.01	0.01
8	0.560	0.547	0.614	0.281	0.318	0.464	0.153	0.068	0.026	0.031	0.040	0.015	0.03	0.01	0.01
9	1.020	1.007	1.071	0.423	0.481	0.800	0.320	0.143	0.032	0.037	0.046	0.021	0.03	0.01	0.01
10	1.109	1.096	0.953	0.743	0.847	0.950	0.158	0.071	0.046	0.051	0.060	0.035	0.05	0.01	0.01
11	1.197	1.184	1.093	0.946	1.078	1.100	0.101	0.045	0.034	0.039	0.048	0.023	0.04	0.01	0.01
12	1.245	1.232	1.235	0.971	1.107	1.158	0.119	0.053	0.031	0.036	0.045	0.020	0.03	0.01	0.01
13	1.379	1.366	1.366	1.098	1.252	1.292	0.120	0.054	0.031	0.036	0.045	0.020	0.03	0.01	0.01
14	1.087	1.074	1.300	1.050	1.280	1.158	0.121	0.054	0.026	0.031	0.040	0.015	0.03	0.01	0.01
15	0.921	0.908	1.253	0.944	1.319	1.069	0.200	0.089	0.029	0.034	0.043	0.018	0.03	0.01	0.01
16	0.921	0.908		1.177	1.342	1.087	0.210	0.105	0.029	0.034	0.043	0.018	0.03	0.01	0.01
17	1.022	1.009		1.105	1.260	1.099	0.115	0.058	0.030	0.035	0.044	0.019	0.03	0.01	0.01
18	0.785	0.772		1.037	1.182	0.944	0.200	0.100	0.032	0.037	0.046	0.021	0.03	0.01	0.01
19	0.677	0.664		1.035	1.180	0.889	0.259	0.130	0.040	0.045	0.054	0.029	0.04	0.01	0.01
20	0.719	0.706		0.988	1.126	0.885	0.207	0.103	0.030	0.035	0.044	0.019	0.03	0.01	0.01
21	0.784	0.758		0.766	0.588	0.724	0.091	0.046	0.036	0.041	0.050	0.025	0.04	0.01	0.01
22	0.520	0.494		0.632	0.605	0.563	0.066	0.033	0.043	0.048	0.057	0.032	0.05	0.01	0.01

Appendix Table 4.93

Adjusted IgM OD values

	Infected								Uninfected						
WPI	SH.11	SH.12	SH. 13	SH. 14	SH. 15	TrMean	StDev	SEMean	SH.16	SH. 17	SH. 18	SH. 19	TrMean	StDev	SEMean
-2	0.052	0.041	0.099	0.043	0.038	0.0546	0.036	0.016	0.017	0.069	0.076	0.031	0.0482	0.0287	0.0144
0	0.067	0.016	0.166	0.083	0.059	0.082	0.051	0.0228	0.085	0.137	0.144	0.099	0.062	0.0287	0.0144
2	0.354	0.303	0.185	0.232	0.143	0.2434	0.086	0.0383	0.111	0.163	0.17	0.125	0.0723	0.0287	0.0144
4	0.382	0.379	0.224	0.252	0.117	0.2708	0.112	0.0501	0.06	0.112	0.119	0.074	0.0913	0.0287	0.0144
6	0.49	0.439	0.218	0.304	0.257	0.3416	0.118	0.0526	0.03	0.082	0.089	0.044	0.0613	0.0287	0.0144
8	0.274	0.223	0.238	0.108	0.488	0.2662	0.139	0.062	0.071	0.123	0.13	0.085	0.1023	0.0287	0.0144
9	0.283	0.232	0.202	0.256	0.473	0.2892	0.107	0.0479	0.037	0.089	0.096	0.051	0.0683	0.0287	0.0144
10	0.237	0.186	0.216	0.233	0.375	0.2494	0.073	0.0327	0.077	0.129	0.136	0.091	0.1082	0.0287	0.0144
11	0.162	0.111	0.178	0.17	0.413	0.2068	0.118	0.0529	0.113	0.165	0.172	0.127	0.1043	0.0287	0.0144
12	0.142	0.091	0.248	0.209	0.29	0.196	0.08	0.0358	0.068	0.12	0.127	0.082	0.0993	0.0287	0.0144
13	0.199	0.148	0.094	0.107	0.34	0.1776	0.1	0.0445	0.097	0.149	0.156	0.111	0.083	0.0287	0.0144
14	0.269	0.218	0.011	0.112	0.326	0.1872	0.126	0.0564	0.009	0.061	0.068	0.023	0.0402	0.0287	0.0144
15	0.136	0.085	0.052	0.189	0.313	0.155	0.103	0.0458	0.07	0.122	0.129	0.084	0.1013	0.0287	0.0144
16	0.051	0		0.167	0.348	0.1415	0.154	0.0772	0.055	0.107	0.114	0.069	0.0862	0.0287	0.0144
17	0.02	-0.031		0.177	0.275	0.1103	0.141	0.0705	0.096	0.148	0.155	0.11	0.073	0.0287	0.0144
18	0.106	0.055		0.091	0.213	0.1163	0.068	0.034	0.11	0.162	0.169	0.124	0.0912	0.0287	0.0144
19	0.144	0.093		0.12	0.237	0.1485	0.063	0.0313	0.079	0.131	0.138	0.093	0.102	0.0287	0.0144
20	0.255	0.204		0.13	0.24	0.2072	0.056	0.0279	0.063	0.115	0.122	0.077	0.0943	0.0287	0.0144
21	0.16	0.109		0.177	0.3	0.1865	0.081	0.0405	0.111	0.163	0.17	0.125	0.1023	0.0287	0.0144
22	0.177	0.126		0.155	0.185	0.1608	0.026	0.0132	0.121	0.173	0.12	0.105	0.1023	0.0287	0.0144

Appendix Table 4.94

Adjusted IgG_2 OD values

	Infected								Uninfected						
WPI	SH.11	SH.12	SH. 13	SH. 14	SH. 15	TrMean	StDev	SEMean	SH.16	SH. 17	SH. 18	SH. 19	TrMean	StDev	SEMean
-2	0.022	0.016	0.027	0.005	0.018	0.056	0.008	0.004	0.018	0.019	0.022	0.022	0.051	0.002	0.001
0	0.024	0.018	0.024	0.006	0.020	0.058	0.007	0.003	0.044	0.013	0.010	0.021	0.054	0.015	0.008
2	0.018	0.012	0.018	0.002	0.014	0.052	0.006	0.003	0.032	0.022	0.038	0.036	0.057	0.007	0.004
4	0.038	0.032	0.027	0.016	0.032	0.068	0.008	0.004	0.020	0.015	0.030	0.028	0.053	0.007	0.004
6	0.057	0.051	0.053	0.029	0.049	0.077	0.022	0.010	0.018	0.017	0.018	0.014	0.050	0.002	0.001
8	0.050	0.044	0.069	0.024	0.043	0.101	0.010	0.004	0.036	0.035	0.038	0.019	0.058	0.009	0.004
9	0.063	0.057	0.052	0.033	0.054	0.091	0.011	0.005	0.029	0.024	0.035	0.014	0.055	0.009	0.004
10	0.066	0.060	0.019	0.035	0.057	0.086	0.020	0.009	0.033	0.035	0.030	0.033	0.058	0.002	0.001
11	0.067	0.061	0.033	0.035	0.058	0.090	0.016	0.007	0.019	0.037	0.013	0.010	0.045	0.012	0.006
12	0.023	0.017	0.010	0.005	0.019	0.048	0.007	0.003	0.025	0.011	0.037	0.010	0.054	0.013	0.006
13	0.027	0.021	0.028	0.008	0.022	0.051	0.008	0.004	0.045	0.035	0.028	0.030	0.054	0.008	0.004
14	0.051	0.045	0.026	0.024	0.044	0.062	0.012	0.005	0.047	0.015	0.037	0.020	0.060	0.015	0.007
15	0.071	0.065	0.024	0.038	0.061	0.071	0.020	0.009	0.032	0.010	0.012	0.030	0.048	0.012	0.006
16	0.048	0.042		0.022	0.041	0.079	0.011	0.006	0.026	0.036	0.022	0.033	0.062	0.006	0.003
17	0.051	0.045		0.024	0.044	0.083	0.012	0.006	0.047	0.019	0.033	0.010	0.053	0.016	0.008
18	0.015	0.009		0.045	0.012	0.066	0.017	0.008	0.039	0.035	0.010	0.037	0.058	0.014	0.007
19	0.063	0.057		0.033	0.054	0.052	0.013	0.007	0.036	0.030	0.010	0.031	0.054	0.011	0.006
20	0.084	0.078		0.047	0.073	0.062	0.016	0.008	0.044	0.014	0.030	0.018	0.059	0.014	0.007
21	0.045	0.045		0.016	0.023	0.064	0.015	0.008	0.043	0.018	0.026	0.031	0.062	0.010	0.005
22	0.055	0.049		0.027	0.047	0.069	0.012	0.006	0.020	0.020	0.021	0.036	0.063	0.008	0.004

Appendix Table 4.95

Adjusted IgA values

	Infected								Uninfected						
WPI	SH.11	SH.12	SH. 13	SH. 14	SH. 15	Mean (+)	StDev	SEMean	SH.16	SH. 17	SH. 18	SH. 19	TrMean	StDev	SEMean
-2	0.022	0.022	0.014	0.020	0.022	0.020	0.005	0.002	0.020	0.007	0.022	0.009	0.015	0.005	0.002
0	0.031	0.032	0.022	0.028	0.032	0.029	0.005	0.002	0.028	0.013	0.031	0.020	0.023	0.005	0.002
2	0.022	0.034	0.032	0.021	0.034	0.029	0.008	0.003	0.021	0.007	0.022	0.008	0.015	0.005	0.002
4	0.005	0.036	0.034	0.023	0.005	0.021	0.011	0.004	0.023	0.005	0.005	0.036	0.017	0.013	0.006
6	0.008	0.032	0.022	0.034	0.008	0.021	0.012	0.005	0.034	0.008	0.008	0.032	0.020	0.010	0.005
8	0.011	0.038	0.028	0.040	0.006	0.025	0.013	0.006	0.040	0.006	0.011	0.038	0.024	0.013	0.006
9	0.012	0.020	0.033	0.008	0.005	0.015	0.013	0.005	0.008	0.005	0.012	0.020	0.011	0.007	0.003
10	0.013	0.025	0.044	0.011	0.019	0.022	0.012	0.005	0.011	0.019	0.013	0.025	0.017	0.003	0.001
11	0.013	0.016	0.035	0.012	0.011	0.017	0.013	0.005	0.012	0.011	0.013	0.016	0.013	0.005	0.002
12	0.012	0.004	0.029	0.013	0.033	0.018	0.008	0.003	0.013	0.033	0.012	0.004	0.016	0.005	0.002
13	0.013	0.013	0.020	0.013	0.044	0.021	0.010	0.004	0.013	0.044	0.013	0.013	0.021	0.005	0.002
14	0.027	0.015	0.024	0.012	0.035	0.023	0.010	0.004	0.012	0.035	0.027	0.015	0.022	0.005	0.002
15	0.038	0.028	0.006	0.013	0.029	0.023	0.013	0.005	0.013	0.029	0.038	0.028	0.027	0.010	0.004
16	0.041	0.032		0.044	0.008	0.031	0.005	0.002	0.044	0.008	0.041	0.032	0.031	0.010	0.005
17	0.026	0.013		0.035	0.010	0.021	0.011	0.005	0.035	0.010	0.026	0.013	0.021	0.005	0.002
18	0.031	0.020		0.029	0.013	0.023	0.012	0.005	0.029	0.013	0.031	0.020	0.023	0.005	0.002
19	0.031	0.020		0.031	0.012	0.024	0.009	0.004	0.031	0.012	0.031	0.020	0.024	0.005	0.002
20	0.027	0.015		0.029	0.011	0.021	0.008	0.004	0.029	0.011	0.027	0.015	0.021	0.005	0.002
21	0.028	0.016		0.033	0.010	0.022	0.008	0.004	0.033	0.010	0.028	0.016	0.022	0.005	0.002
22	0.018	0.004		0.033	0.008	0.016	0.009	0.004	0.033	0.008	0.018	0.004	0.016	0.008	0.004

Experiment three (4): Adjusted values for *F. gigantica* (Kenyan strain) infected and uninfected sheep

Appendix Table 4.96

Adjusted total Ig OD values

WPI	sh. 23	sh. 25	sh. 27	sh. 29	sh. 21	sh. 31
-2	-0.069	-0.065	-0.094	-0.048	-0.068	0.068
0	-0.035	-0.010	0.084	-0.054	0.095	0.032
2	0.056	-0.030	0.029	0.077	-0.052	0.061
4	-0.018	-0.016	0.256	0.086	0.053	0.090
6	0.096	0.291	0.260	0.252	0.046	-0.056
8	1.000	1.036	0.489	0.628	0.027	-0.068
9	0.465	1.155	0.608	0.915	0.009	0.052
10	1.016	1.284	0.911	0.679	0.087	0.057
11	1.185	1.301	1.119	0.826	0.055	-0.008
12	1.300	1.453	1.128	1.275	0.019	-0.040
13	1.310	1.382	1.217	1.097	0.063	0.018
14	1.257	1.279	1.144	1.095	0.029	0.028
15	1.313	1.100	1.226	1.230	0.009	0.074
16		1.149	1.217	1.098	0.009	0.133
17			1.047	0.991	-0.042	0.013
18			1.206	1.027	0.012	-0.007
19			1.118	0.962	0.096	0.020
20			1.065	1.290	0.065	0.020
21			0.729	0.788	0.019	0.055
22			0.400	0.741	-0.088	-0.002

Adjusted IgG_1 OD values

WPI	sh. 23	sh. 25	sh. 27	sh. 29	sh. 21	sh. 31
-2	-0.031	0.101	-0.006	-0.023	0.025	-0.017
0	0.040	0.056	-0.040	-0.016	0.016	-0.018
2	0.108	0.084	-0.007	-0.007	0.021	-0.011
4	0.366	0.055	-0.019	-0.003	0.015	0.002
6	0.355	-0.011	-0.017	0.034	0.015	-0.008
8	0.345	0.068	0.053	0.169	-0.002	-0.008
9	0.256	0.065	0.354	0.357	0.001	-0.006
10	0.367	0.121	0.361	0.278	0.010	-0.005
11	0.400	0.093	0.455	0.440	0.005	-0.005
12	0.268	0.213	0.471	0.648	0.007	0.007
13	0.181	0.117	0.599	0.383	0.003	-0.009
14	0.093	0.089	0.931	0.144	0.001	-0.001
15	0.103	0.056	0.136	0.286	0.001	0.001
16		0.027	0.126	0.185	0.003	-0.007
17			0.182	0.092	0.012	0.001
18			0.008	0.135	-0.005	-0.011
19			-0.014	0.109	-0.005	-0.004
20			0.111	0.118	0.005	0.003
21			0.135	0.152	-0.005	0.001
22			0.191	0.123	0.001	0.007

Appendix Table 4.97

Adjusted IgM OD values

WPI	sh. 23	sh. 25	sh. 27	sh. 29	sh. 21	sh. 31
-2	0.034	0.026	0.001	0.046	0.004	0.020
0	0.034	0.023	0.054	0.039	0.002	0.027
2	0.123	0.075	0.235	0.207	0.005	0.024
4	0.056	0.051	0.207	0.239	0.003	0.022
6	0.089	0.040	0.276	0.172	0.003	0.016
8	0.100	0.070	0.079	0.160	-0.001	0.003
9	0.074	0.054	0.041	0.176	0.002	0.023
10	0.061	0.040	0.237	0.153	0.002	0.018
11	0.012	0.034	0.208	0.182	0.001	0.017
12	0.183	0.012	0.212	0.191	0.000	0.020
13	0.011	0.004	0.161	0.143	-0.002	0.009
14	0.106	-0.010	0.266	0.092	0.010	0.012
15	0.079	-0.004	0.224	0.153	0.000	0.022
16		-0.012	0.196	0.240	-0.002	0.033
17			0.214	0.267	-0.004	0.031
18			0.222	0.142	0.001	0.009
19			0.250	0.136	0.022	0.009
20			0.186	0.129	0.005	0.010
21			0.106	0.133	0.001	0.033
22			0.055	0.097	-0.001	0.025

Adjusted IgG_2 OD values

WPI	sh. 23	sh. 25	sh. 27	sh. 29	sh. 21	sh. 31
-2	0.011	0.008	0.023	-0.019	0.006	-0.001
0	0.014	0.016	0.041	-0.012	0.005	0.002
2	0.008	0.026	0.080	-0.012	0.009	0.002
4	-0.005	-0.002	0.076	-0.012	0.004	0.003
6	-0.002	0.019	0.047	-0.014	0.003	-0.001
8	0.008	0.001	0.004	-0.012	-0.005	-0.001
9	0.006	-0.002	0.006	-0.012	-0.007	-0.001
10	0.004	0.000	0.054	-0.014	-0.003	0.001
11	0.003	0.003	0.049	-0.010	-0.001	-0.002
12	0.008	0.010	0.068	-0.008	-0.005	-0.004
13	0.001	0.013	0.035	-0.006	-0.010	-0.003
14	0.006	0.001	0.039	-0.006	-0.007	-0.003
15	0.010	0.001	0.031	-0.008	-0.005	0.001
16		0.004	0.035	-0.010	-0.010	-0.007
17			0.070	-0.008	-0.010	0.002
18			0.043	-0.010	-0.006	0.000
19			0.054	-0.010	-0.007	-0.002
20			0.047	-0.010	-0.004	0.004
21			-0.004	-0.012	-0.006	0.008
22			-0.010	-0.010	-0.005	0.003

Appendix Table 4.98

Adjusted IgA values

WPI	sh. 23	sh. 25	sh. 27	sh. 29	sh. 21	sh. 31
-2	0.012	-0.010	0.015	0.004	0.020	0.024
0	0.036	0.010	0.014	0.003	0.030	0.032
2	0.049	0.036	0.016	0.001	0.018	0.021
4	-0.006	-0.012	0.016	-0.001	0.014	0.029
6	0.007	-0.001	0.023	0.002	0.013	0.023
8	-0.006	-0.008	0.008	0.001	0.013	0.019
9	-0.008	-0.010	-0.001	0.001	0.009	0.023
10	-0.001	-0.008	0.003	-0.001	0.007	0.019
11	0.001	-0.006	0.002	-0.002	-0.001	0.011
12	0.007	0.003	0.006	0.002	0.006	0.014
13	-0.012	-0.001	0.002	-0.001	0.011	0.018
14	-0.010	-0.004	0.003	0.003	0.015	0.027
15	-0.006	-0.004	0.002	0.002	0.010	0.018
16		-0.001	0.001	0.001	0.004	0.018
17			0.002	0.012	0.003	0.034
18			0.008	0.008	0.015	0.016
19			0.020	0.007	0.014	0.048
20			0.012	0.007	0.008	0.025
21			-0.001	0.011	0.006	0.028
22			-0.003	0.002	0.004	0.048

Table 4.99 Adjusted total Ig OD values of *F. hepatica* and *F. gigantica infected calves*

F. hepatica							F. gigantica				
WPI	14c	15c	23c	26c	34c	45c	WPI	22	23	24	26
-27	0.007	0.028	0.088	0.049			-2	0.045	-0.028	0.009	0.059
-26	0.002	0.056	0.083	0.005			-1	0.002	-0.032	0.026	0.004
-25	0.006	0.144	0.053	0.024			0	0.033	-0.025	0.040	0.028
-24	0.024	-0.015	-0.003	-0.005			1	-0.016	-0.047	0.023	-0.009
-23	0.012	0.024	-0.010	-0.008			2	-0.019	-0.041	0.012	-0.013
-22	0.054	-0.012	-0.004	-0.004			3	-0.027	-0.018	0.029	-0.008
-21	0.551	0.025	-0.031	-0.001			4	-0.018	0.005	0.023	-0.004
-20	1.047	0.085	-0.036	-0.012			5	0.045	0.046	0.053	-0.018
-19	1.141	0.166	-0.019	-0.030			6	0.002	0.033	0.047	-0.040
-18	1.063	1.415	-0.043	-0.029			7	0.018	0.542	0.395	-0.039
-17	1.350	1.561	-0.016	-0.015			8	0.016	0.785	0.346	-0.021
-16	1.337	0.763	-0.039	-0.034			9	0.066	0.710	0.510	-0.045
-15	1.165	1.115	-0.043	-0.027			10	0.455	0.880	0.760	-0.036
-14	0.992	1.389	-0.029	-0.026			11	0.442	0.944	0.825	-0.035
-13	0.687	1.193	-0.023	-0.021			12	0.261	1.106	0.870	-0.029
-12	0.806	1.073	-0.013	-0.025			13	0.844	1.137	0.759	-0.034
-11	0.679	0.786	0.019	-0.017			14	0.792	1.219	1.032	-0.024
-10	0.830	0.554	0.156	-0.024			15	0.867	1.189	1.068	-0.033
-9	0.428	0.401	0.473	-0.027			16	0.939	1.261	1.170	-0.036
-8	0.312	0.488	1.166	-0.039			17	0.762	1.212	1.130	-0.051
-7	0.550	0.576	1.491	-0.032			18	0.731	1.126	1.067	-0.043
-6	0.420	0.262	1.508	-0.019			19	0.948	0.895	1.109	-0.026
-5	0.348	0.314	1.624	-0.019			20	0.754	1.091	1.112	-0.026
-4	0.274	0.183	1.618	-0.010			21	0.721	1.100	1.080	-0.015
-3	0.213	0.329	1.585	0.022			22	0.607	1.218	1.192	-0.043
-2	0.434	0.347	1.381	-0.015			23	0.697	1.214	1.030	-0.026
-1	0.318	0.432	1.514	-0.021			24	0.673	0.918	0.923	-0.026
0	0.230	0.306	1.555	-0.021	0.008	-0.038	25	0.575	1.091	1.113	-0.015
1	0.154	0.301	1.510	-0.022	0.030	-0.016	26	0.615	1.132	1.053	0.025
2	0.137	0.421	1.475	-0.005	0.015	-0.014	27	0.537	1.104	1.281	-0.021
3	0.162	0.498	1.640	-0.021	0.068	-0.013	28	0.552	1.060	1.139	-0.029
4	0.171	0.280	1.310	-0.012	0.689	0.027	29	0.468	1.027	1.072	-0.029
5	0.173	0.243	1.368	-0.015	1.309	0.094	30	0.456	1.105	1.038	-0.030
6	0.150	0.287	1.160	-0.003	1.426	0.183	31	0.347	1.108	0.883	-0.009
7	0.108	0.345	1.496	0.005	1.329	1.558	32	0.333	0.948	0.759	-0.029
8	0.159	0.654	1.303	-0.016	1.688	1.719					
9	0.120	0.699	0.901	-0.013	1.671	0.840					
10	0.059	0.405	0.930	-0.012		1.227					
11	0.104	0.332	1.108	-0.015							
12	0.216	0.314	0.673	-0.003							

Table 4.100 Adjusted IgG1 OD values of *F. hepatica* and *F. gigantica* infected calves

F. hepatica							F. gigantica				
WPI	14c	15c	23c	26c	34c	45c	WPI	22	23	24	26
-27	0.017	0.065	0.165	0.142			-2	0.156	-0.022	0.045	0.165
-26	-0.008	0.142	0.155	0.126			-1	0.063	-0.004	0.149	0.147
-25	0.034	0.259	0.084	0.116			0	0.076	-0.017	0.123	0.135
-24	0.057	-0.040	-0.023	0.145			1	0.048	-0.029	0.118	0.168
-23	0.013	0.141	-0.041	0.095			2	0.053	-0.029	0.079	0.111
-22	0.204	-0.019	-0.041	0.025			3	0.038	-0.002	0.130	0.029
-21	0.942	0.079	-0.092	0.070			4	0.045	0.011	0.085	0.081
-20	1.161	0.208	-0.117	0.028			5	0.085	0.067	0.221	0.033
-19	1.161	0.329	-0.101	0.047			6	0.100	0.050	0.282	0.054
-18	1.084	1.349	-0.091	-0.017			7	0.139	0.475	1.161	-0.020
-17	1.441	1.459	-0.086	-0.021			8	0.075	1.178	0.960	-0.025
-16	1.359	1.144	-0.140	0.065			9	0.133	1.014	1.393	0.076
-15	1.190	1.386	-0.118	-0.033			10	0.678	1.060	1.425	-0.039
-14	0.999	1.302	-0.014	-0.013			11	0.584	0.816	1.477	-0.015
-13	0.942	1.397	-0.050	0.007			12	0.477	1.072	1.613	0.008
-12	1.222	1.390	-0.050	-0.034			13	1.381	1.671	1.408	-0.040
-11	0.940	0.812	-0.062	-0.015			14	1.487	1.646	1.779	-0.018
-10	0.812	0.836	0.257	-0.049			15	1.334	1.597	1.681	-0.057
-9	0.742	0.671	0.841	-0.037			16	1.226	1.273	1.730	-0.044
-8	0.625	0.899	1.162	-0.014			17	1.181	1.199	1.599	-0.016
-7	0.889	0.906	1.144	-0.050			18	1.381	1.577	1.674	-0.058
-6	0.571	0.521	1.117	-0.019			19	1.531	1.265	1.662	-0.022
-5	0.670	0.468	0.920	-0.025			20	1.226	1.127	1.714	-0.029
-4	0.594	0.210	0.887	0.009			21	0.943	1.046	1.769	0.005
-3	0.331	0.636	1.320	0.026			22	1.266	1.246	1.761	0.030
-2	0.806	0.701	1.227	-0.043			23	1.252	1.632	1.597	-0.050
-1	0.525	0.571	1.067	-0.013			24	1.291	1.344	1.663	-0.015
0	0.417	0.530	1.102	-0.039	0.034	-0.096	25	1.196	1.239	1.714	-0.046
1	0.399	0.462	1.003	-0.031	0.057	-0.126	26	1.226	1.163	1.718	-0.036
2	0.333	0.762	1.084	-0.006	0.013	-0.108	27	0.995	1.005	1.792	-0.007
3	0.605	0.715	1.227	-0.024	0.204	-0.022	28	1.083	1.489	1.734	-0.028
4	0.436	0.373	1.165	0.048	0.942	0.086	29	1.083	1.133	1.735	0.055
5	0.413	0.427	0.873	0.059	1.161	0.228	30	1.039	1.061	1.753	0.068
6	0.388	0.635	1.238	0.016	1.161	0.360	31	0.854	0.950	1.561	0.019
7	0.306	0.712	1.341	0.024	1.084	1.481	32	0.846	0.649	0.487	0.028
8	0.483	1.114	1.120	-0.038	1.441	1.602					
9	0.417	1.017	1.193	-0.099	1.359	1.256					
10	0.213	0.752	1.121	-0.057	1.190	1.522					
11	0.316	0.678	1.058	-0.077		1.430					
12	0.449	0.872	1.076	-0.076							

Table4.101 Adjusted IgM OD values of *F. hepatica* and *F. gigantica* infected calves

F. hepatica							*F. gigantica*				
WPI	14c	15c	23c	26c	34c	45c	**WPI**	22	23	24	26
-27	0.017	0.049	-0.023	0.057			**-2**	0.035	-0.03	0.101	0.109
-26	0.050	0.084	-0.039	0.023			**-1**	0.134	0.009	0.126	0.018
-25	0.045	0.067	0.032	0.045			**0**	0.161	0.031	0.12	0.078
-24	0.084	0.026	0.028	0.046			**1**	0.228	0.197	0.155	0.080
-23	0.114	0.264	-0.023	0.038			**2**	0.374	0.031	0.35	0.060
-22	0.288	0.392	-0.039	0.058			**3**	0.669	0.584	0.978	0.111
-21	0.343	0.151	0.036	0.083			**4**	1.134	0.733	0.725	0.058
-20	0.238	0.138	-0.012	0.079			**5**	0.644	0.717	0.678	0.068
-19	0.284	0.107	-0.021	0.108			**6**	0.675	0.468	0.656	0.046
-18	0.253	0.064	-0.043	0.054			**7**	0.534	0.405	0.815	0.101
-17	0.254	0.112	0.012	0.068			**8**	0.6	0.301	0.387	0.137
-16	0.493	0.075	0.176	0.020			**9**	0.462	0.465	0.389	0.010
-15	0.283	0.079	0.258	0.065			**10**	0.636	0.447	0.762	0.131
-14	0.347	0.115	0.261	0.082			**11**	0.383	0.52	0.833	0.175
-13	0.426	0.218	0.167	0.064			**12**	0.356	0.721	0.906	0.127
-12	0.493	0.598	0.402	0.076			**13**	0.467	0.146	0.606	0.159
-11	0.416	1.145	0.466	0.181			**14**	0.486	0.362	1.112	0.040
-10	0.463	0.938	0.431	0.038			**15**	0.345	0.252	0.455	0.058
-9	0.415	0.394	0.482	0.003			**16**	0.243	0.468	0.791	-0.034
-8	0.398	0.502	0.422	0.071			**17**	0.363	0.761	0.876	0.145
-7	0.524	0.338	0.543	0.055			**18**	0.873	0.691	0.735	0.103
-6	0.325	0.106	0.414	0.108			**19**	0.299	0.769	0.563	0.131
-5	0.441	0.109	0.509	0.115			**20**	0.591	0.69	0.779	0.175
-4	0.518	0.046	0.641	0.157			**21**	0.225	0.355	0.731	0.127
-3	0.188	0.510	0.644	0.173			**22**	0.342	1.019	0.719	0.159
-2	0.961	0.214	0.616	0.147			**23**	0.281	0.286	0.456	0.040
-1	0.731	0.277	0.810	0.068			**24**	0.29	0.396	0.567	0.139
0	0.539	0.190	0.757	0.078	0.030	0.079	**25**	0.107	0.489	0.533	0.165
1	0.358	0.145	0.665	0.080	0.150	0.444	**26**	0.225	0.583	0.702	0.169
2	0.378	0.273	0.662	0.187	0.331	0.233	**27**	0.425	0.763	1.214	0.159
3	0.460	0.367	0.858	0.221	0.334	0.425	**28**	0.429	0.773	0.965	0.040
4	0.392	0.146	0.797	0.160	0.220	0.485	**29**	0.438	0.671	0.851	0.139
5	0.411	0.105	0.480	0.179	0.507	0.629	**30**	0.297	0.587	0.833	0.034
6	0.301	0.179	0.582	0.125	0.585	0.420	**31**	0.083	0.63	0.701	0.090
7	0.352	0.152	0.517	0.216	0.542	0.667	**32**	0.227	0.261	0.684	0.133
8	0.426	0.218	0.256	0.140	0.605	0.576					
9	0.373	0.217	0.394	0.151	0.531	0.650					
10	0.178	0.160	0.617	0.076	0.679	0.746					
11	0.436	0.118	0.582	0.099		0.906					
12	0.533	0.223	0.445	0.107							

Table 4.102 Adjusted IgG_2 OD values of *F. hepatica* and *F. gigantica* infected calves

F. hepatica							*F. gigantica*				
WPI	14c	15c	23c	26c	34c	45c	**WPI**	22	23	24	26
-27	0.021	0.012	0.009	0.008			**-2**	0.027	-0.032	-0.038	0.033
-26	-0.025	0.046	0.011	0.001			**-1**	0.032	0.087	0.060	0.025
-25	0.021	0.020	0.042	0.005			**0**	0.004	0.079	0.035	0.029
-24	0.032	0.026	0.004	0.017			**1**	0.017	0.091	0.037	0.043
-23	0.008	0.037	0.019	0.006			**2**	0.067	0.072	0.052	0.031
-22	0.002	0.002	-0.020	0.006			**3**	0.280	0.086	0.022	0.031
-21	-0.012	0.007	-0.020	0.026			**4**	0.193	0.049	-0.026	0.053
-20	0.014	-0.008	-0.024	-0.030			**5**	0.269	0.044	0.000	-0.009
-19	0.031	0.014	-0.026	0.005			**6**	0.162	-0.003	0.005	0.029
-18	0.038	0.050	-0.003	-0.012			**7**	0.197	0.101	0.068	0.011
-17	0.114	0.077	0.042	0.003			**8**	0.281	0.085	-0.037	-0.017
-16	0.075	0.007	-0.022	0.009			**9**	0.193	0.049	0.041	-0.011
-15	0.044	0.003	-0.014	-0.018			**10**	0.193	0.144	0.139	0.004
-14	0.017	0.053	0.016	0.006			**11**	0.191	0.187	0.146	0.031
-13	0.037	0.058	0.047	-0.022			**12**	0.223	0.296	0.140	0.000
-12	-0.002	0.039	0.027	0.010			**13**	0.358	0.292	0.195	0.036
-11	0.028	0.076	0.008	0.042			**14**	0.277	0.355	0.323	0.071
-10	0.037	0.012	0.134	-0.021			**15**	0.307	0.331	0.259	0.001
-9	0.028	0.015	0.104	-0.024			**16**	0.211	0.409	0.356	-0.003
-8	0.033	0.038	0.104	0.003			**17**	0.210	0.414	0.276	0.028
-7	0.069	0.036	0.230	0.036			**18**	0.223	0.237	0.340	0.064
-6	0.129	-0.004	0.251	0.010			**19**	0.214	0.400	0.195	0.052
-5	0.169	0.015	0.496	0.046			**20**	0.219	0.415	0.211	0.076
-4	0.204	-0.006	0.397	0.014			**21**	0.160	0.231	0.227	0.040
-3	0.375	0.038	0.341	0.037			**22**	0.222	0.358	0.361	0.065
-2	0.108	0.346	0.263	0.070			**23**	0.231	0.134	0.220	0.101
-1	0.116	0.589	0.342	-0.031			**24**	0.197	0.130	0.326	-0.011
0	0.056	0.558	0.279	0.013	-0.039	0.025	**25**	0.234	0.225	0.264	0.038
1	0.024	0.326	0.286	0.022	-0.046	0.032	**26**	0.167	0.204	0.223	0.049
2	0.039	0.586	0.235	0.022	0.008	0.046	**27**	0.183	0.230	0.425	0.049
3	0.058	0.277	0.239	0.078	0.002	0.002	**28**	0.205	0.181	0.326	0.111
4	0.037	0.160	0.228	0.030	-0.012	0.009	**29**	0.219	0.135	0.166	0.058
5	0.071	0.140	0.307	0.056	0.014	-0.010	**30**	0.196	0.214	0.181	0.087
6	0.025	0.174	0.122	0.027	0.031	0.018	**31**	0.204	0.245	0.023	0.054
7	0.038	0.112	0.092	0.039	0.038	0.062	**32**	0.194	0.170	-0.004	0.068
8	0.190	0.165	0.117	-0.002	0.113	0.096					
9	0.120	0.223	0.114	-0.005	0.074	0.009					
10	0.050	0.117	0.093	-0.056		0.003					
11	0.094	0.081	0.155	0.032							
12	0.173	0.151	0.062	0.062							

Table 4.103 Adjusted IgA OD values of *F. hepatica* and *F. gigantica* infected calves

F. hepatica							F. gigantica				
WPI	14c	15c	23c	26c	34c	45c	WPI	22	23	24	26
-27	0.063	0.017	-0.002	0.015							
-26	-0.008	0.007	-0.007	0.015			-2	0.032	0.019	0.017	0.038
-25	0.032	0.018	0.018	-0.029			-1	0.044	0.019	0.043	0.038
-24	0.058	0.011	-0.016	-0.021			0	0.033	0.025	0.019	0.010
-23	0.015	0.009	0.002	-0.011			1	0.031	0.026	0.015	0.015
-22	0.028	0.020	-0.023	-0.004			2	0.031	0.043	0.015	0.022
-21	0.074	0.014	-0.043	0.028			3	0.046	0.037	0.047	0.026
-20	0.152	0.009	-0.036	0.000			4	0.022	0.033	-0.004	0.046
-19	0.107	-0.006	-0.005	-0.011			5	0.012	0.019	-0.026	0.029
-18	0.065	0.156	-0.011	0.004			6	0.021	0.018	-0.006	0.022
-17	0.149	0.218	0.009	-0.003			7	0.054	0.065	0.051	0.031
-16	0.119	0.041	-0.007	0.013			8	0.054	0.103	0.086	0.027
-15	0.072	0.101	0.014	0.000			9	0.070	0.071	0.083	0.037
-14	0.056	0.126	0.041	-0.001			10	0.065	0.082	0.105	0.029
-13	0.011	0.161	0.047	-0.006			11	0.103	0.111	0.090	0.028
-12	0.098	0.117	0.072	0.007			12	0.102	0.147	0.150	0.025
-11	0.070	0.258	0.022	0.020			13	0.105	0.151	0.208	0.034
-10	0.067	0.137	0.036	-0.031			14	0.083	0.144	0.201	0.041
-9	0.126	0.032	0.094	-0.023			15	0.173	0.147	0.111	0.009
-8	0.031	0.026	0.207	-0.030			16	0.079	0.155	0.180	0.014
-7	0.091	0.060	0.315	0.013			17	0.174	0.153	0.210	0.010
-6	0.051	0.009	0.275	0.061			18	0.163	0.158	0.205	0.037
-5	0.095	-0.002	0.311	0.024			19	0.102	0.101	0.158	0.068
-4	0.017	-0.006	0.302	-0.019			20	0.099	0.118	0.152	0.044
-3	0.061	0.026	0.250	-0.004			21	0.072	0.131	0.184	0.017
-2	0.082	0.024	0.297	-0.014			22	0.089	0.189	0.193	0.026
-1	0.100	0.033	0.301	-0.013			23	0.102	0.182	0.270	0.020
0	0.008	0.018	0.230	0.005	-0.017	0.017	24	0.073	0.155	0.169	0.021
1	0.001	-0.005	0.227	-0.015	-0.026	0.001	25	0.078	0.128	0.167	0.032
2	-0.014	0.035	0.221	-0.013	-0.015	0.003	26	0.140	0.132	0.173	0.019
3	0.010	0.024	0.293	-0.009	0.000	0.064	27	0.127	0.135	0.126	0.021
4	0.004	0.012	0.236	-0.008	0.093	0.110	28	0.185	0.160	0.259	0.023
5	0.010	-0.003	0.211	-0.006	0.126	0.187	29	0.143	0.139	0.197	0.024
6	0.021	0.029	0.214	-0.023	0.123	0.143	30	0.188	0.146	0.190	0.025
7	-0.012	0.041	0.275	0.009	0.091	0.101	31	0.150	0.155	0.118	0.014
8	0.068	0.086	0.270	-0.021	0.077	0.184	32	0.171	0.121	0.188	0.034
9	0.026	0.099	0.211	-0.023	0.128	0.154					
10	-0.001	0.035	0.184	-0.020	0.081	0.108					
11	0.000	0.065	0.279	-0.024		0.092					
12	0.053	0.069	0.193	-0.025							

Appendix Table 4

Optical Density titration for ELISA using *Fasciola hepatica* Glutathion S-Transferase (FhGST).

Appendix Table 4.104

Titrationsof total Ig showing mean OD values obtained in *F. hepatica* and *F. gigantica* infected calves.

Antigen (mg/ml)

F. hepatica.					*F. gigantic*				
FhGST	B	N	P1	P2	FhGST	B	N	P1	P2
2	0.083	0.250	0.745	0.842	2	0.114	0.136	0.432	0.412
1	0.064	0.181	0.642	0.754	1	0.183	0.141	0.445	0.315
0.5	0.057	0.190	0.516	0.808	0.5	0.087	0.134	0.385	0.421

Serum

F. hepatica.					*F. gigantic*				
Serun	B	N	P1	P2	Serun	B	N	P1	P2
25	0.0833	0.40525	0.74475	0.84175	25	0.114	0.356	0.351	0.551
50	0.064	0.181	0.642	0.554	50	0.1083	0.199	0.245	0.422
100	0.05725	0.09025	0.216	0.308	100	0.08675	0.141	0.185	0.27
200	0.0285	0.072	0.13725	0.192	200	0.084	0.102	0.15	0.19
400	0.072	0.059	0.09575	0.14	400	0.071	0.09	0.096	0.148
800	0.051	0.04275	0.064	0.086	800	0.092	0.069	0.076	0.118

Conjugate

F. hepatica.					*F. gigantic*				
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.038	0.101	0.177	0.266	1000	0.035	0.075	0.184	0.315
2000	0.025	0.052	0.101	0.149	2000	0.022	0.037	0.071	0.181
4000	0.017	0.039	0.061	0.104	4000	0.020	0.019	0.050	0.096
8000	0.016	0.025	0.032	0.063	8000	0.016	0.015	0.035	0.059
16000	0.018	0.005	0.016	0.043	16000	0.017	0.003	0.026	0.045
32000	0.012	0.001	0.008	0.024	32000	0.021	-0.001	0.010	0.027

Appendix Table 4.105

Titration of IgG1 Showing mean OD values obtained in *F. hepatica* and *F. gigantica* infected calves.

Antigen (mg/ml)

F. hepatica.					*F. gigantic*				
FhGST	B	N	P1	P2	FhGST	B	N	P1	P2
2	0.1011	0.0500	0.6100	0.6500	2	0.0833	0.4205	0.0750	0.6750
1	0.0900	0.0300	0.6230	0.5900	1	0.0640	0.4810	0.6420	0.5540
0.5	0.0894	0.4120	0.7980	0.6870	0.5	0.0573	0.4025	0.6000	0.5800

Serum

F. hepatica.					*F. gigantic*				
Serum	B	N	P1	P2	Serum	B	N	P1	P2
50	0.0110	0.0500	0.6100	0.6500	50	0.0833	0.40525	0.74475	0.84175
100	0.0900	0.0300	0.2300	0.5900	100	0.064	0.181	0.642	0.554
200	0.0100	0.0600	0.1900	0.1500	200	0.05725	0.09025	0.216	0.308
400	0.0080	0.0490	0.2400	0.3300	400	0.0285	0.072	0.13725	0.192
800	0.0070	0.0300	0.2000	0.3100	800	0.072	0.059	0.09575	0.14
1600	0.0080	0.0270	0.1400	0.2500	1600	0.051	0.04275	0.064	0.086

Conjugate

F. hepatica.					*F. gigantic*				
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.214	0.412	0.798	0.687	1000	0.100	0.223	0.694	0.583
2000	0.165	0.161	0.632	0.480	2000	0.087	0.122	0.528	0.376
4000	0.147	0.138	0.379	0.312	4000	0.064	0.109	0.275	0.208
8000	0.131	0.121	0.227	0.249	8000	0.044	0.067	0.123	0.145
16000	0.111	0.157	0.205	0.187	16000	0.032	0.068	0.101	0.083
32000	0.101	0.124	0.168	0.142	32000	0.024	0.035	0.064	0.038

Appendix Table 4.106

Titration of IgM Showing mean OD values obtained in *F. hepatica* and *F. gigantica* infected calves.

Antigen (mg/ml)

F. hepatica.					*F. gigantic*				
FhGST	B	N	P1	P2	FhGST	B	N	P1	P2
2	0.106	0.295	0.872	0.642	2	0.087	0.387	0.483	0.527
1	0.084	0.264	0.887	0.594	1	0.081	0.288	0.614	0.668
0.5	0.076	0.211	0.614	0.541	0.5	0.064	0.316	0.414	0.451

Serum

F. hepatica.					*F. gigantic*				
Serum	B	N	P1	P2	Serum	B	N	P1	P2
50	0.106	0.295	0.872	0.642	50	0.087	0.387	0.983	0.727
100	0.084	0.264	0.887	0.594	100	0.081	0.288	0.614	0.668
200	0.076	0.211	0.614	0.411	200	0.064	0.159	0.414	0.451
400	0.053	0.224	0.340	0.204	400	0.056	0.169	0.330	0.301
800	0.037	0.180	0.228	0.190	800	0.064	0.146	0.191	0.211
1600	0.220	0.144	0.161	0.183	1600	0.061	0.105	0.176	0.209

Conjugate

F. hepatica.					*F. gigantic*				
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.179	0.318	0.913	0.709	1000	0.084	0.252	0.910	0.725
2000	0.159	0.229	0.510	0.398	2000	0.102	0.215	0.500	0.583
4000	0.119	0.218	0.421	0.259	4000	0.098	0.124	0.891	0.625
8000	0.083	0.201	0.336	0.215	8000	0.77	0.119	0.215	0.213
16000	0.046	0.188	0.201	0.192	16000	0.068	0.098	0.154	0.125
32000	0.022	0.096	0.117	0.184	32000	0.520	0.078	0.097	0.078

Appendix Table 4.107

Titrations of IgG2 Showing mean OD values obtained in *F. hepatica* and *F. gigantica* infected calves.

Antigen (mg/ml)

F. hepatica.					*F. gigantic*				
FhGST	B	N	P1	P2	FhGST	B	N	P1	P2
2	0.043	0.102	0.201	0.380	2	0.087	0.092	0.198	0.109
1	0.037	0.119	0.260	0.298	1	0.054	0.065	0.107	0.109
0.5	0.028	0.082	0.391	0.277	0.5	0.053	0.038	0.192	0.161

Serum

F. hepatica.					*F. gigantic*				
Serum	B	N	P1	P2	Serum	B	N	P1	P2
50	0.043	0.102	0.244	0.108	50	0.087	0.092	0.198	0.087
100	0.037	0.119	0.126	0.098	100	0.054	0.065	0.107	0.088
200	0.028	0.082	0.091	0.077	200	0.053	0.038	0.092	0.061
400	0.022	0.031	0.074	0.056	400	0.034	0.021	0.064	0.040
800	0.013	0.020	0.061	0.035	800	0.018	0.011	0.034	0.038
1600	0.006	0.010	0.049	0.029	1600	0.005	0.008	0.018	0.024

Conjugate

F. hepatica.					*F. gigantic*				
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.031	0.033	0.060	0.095	1000	0.027	0.063	0.099	0.098
2000	0.028	0.030	0.050	0.035	2000	0.020	0.052	0.071	0.091
4000	0.022	0.025	0.022	0.031	4000	0.014	0.044	0.064	0.058
8000	0.020	0.026	0.027	0.033	8000	0.012	0.041	0.022	0.049
16000	0.029	0.030	0.020	0.029	16000	0.006	0.043	0.019	0.028
32000	0.011	0.031	0.030	0.021	32000	0.003	0.056	0.008	0.011

Appendix Table 4.108

Titrations of IgA Showing mean OD values obtained in *F. hepatica* and *F. gigantica* infected calves.

Antigen (mg/ml)

F. hepatica.					*F. gigantic*				
FhGST	B	N	P1	P2	FhGST	B	N	P1	P2
2	0.032	0.078	0.253	0.157	2	0.039	0.045	0.113	0.157
1	0.029	0.041	0.208	0.158	1	0.033	0.039	0.108	0.158
0.5	0.012	0.040	0.100	0.093	0.5	0.024	0.040	0.101	0.108

Serum

F. hepatica.					*F. gigantic*				
Serum	B	N	P1	P2	Serum	B	N	P1	P2
50	0.032	0.078	0.253	0.157	50	0.039	0.045	0.113	0.157
100	0.029	0.041	0.208	0.158	100	0.033	0.039	0.108	0.158
200	0.012	0.040	0.100	0.093	200	0.024	0.040	0.100	0.093
400	0.011	0.032	0.070	0.054	400	0.250	0.026	0.076	0.054
800	0.009	0.025	0.049	0.032	800	0.019	0.021	0.029	0.032
1600	0.004	0.037	0.058	0.025	1600	0.014	0.017	0.022	0.025

Conjugate

F. hepatica.					*F. gigantic*				
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.031	0.033	0.060	0.095	1000	0.027	0.063	0.079	0.099
2000	0.028	0.030	0.050	0.085	2000	0.020	0.052	0.065	0.087
4000	0.022	0.025	0.022	0.031	4000	0.014	0.044	0.042	0.072
8000	0.020	0.026	0.027	0.033	8000	0.012	0.041	0.022	0.053
16000	0.029	0.030	0.020	0.031	16000	0.006	0.043	0.019	0.049
32000	0.011	0.031	0.030	0.029	32000	0.003	0.056	0.008	0.028

Appendix Table 4.109

Titration of total Ig Showing mean OD values obtained in *F. hepatica* and *F. gigantica* infected sheep

Antigen (μg/ml)

F. hepatica.					*F. gigantic*				
FhGST	B	N	P1	P2	FhGST	B	N	P1	P2
2	0.117	0.161	0.278	0.367	2	0.112	0.156	0.450	0.433
1	0.124	0.131	0.495	0.348	1	0.183	0.141	0.500	0.305
0.5	0.105	0.141	0.252	0.434	0.5	0.087	0.134	0.385	0.207

Serum

F. hepatica. *F. gigantic*

Serum	B	N	P1	P2	Serum	B	N	P1	P2
25	0.06	0.492	0.645	0.59	25	0.061	0.565	0.73	0.77
50	0.058	0.33	0.423	0.471	50	0.056	0.406	0.567	0.553
100	0.054	0.265	0.281	0.27	100	0.06	0.26	0.384	0.359
200	0.055	0.208	0.22	0.228	200	0.065	0.206	0.248	0.274
400	0.076	0.14	0.165	0.157	400	0.056	0.175	0.158	0.18
800	0.077	0.112	0.114	0.116	800	0.05	0.089	0.125	0.145

Conjugate

F. hepatica.					*F. gigantic*				
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.042	0.392	0.687	0.59	1000	0.071	0.565	0.795	0.57
2000	0.035	0.333	0.623	0.471	2000	0.06	0.406	0.67	0.453
4000	0.028	0.274	0.359	0.352	4000	0.04	0.247	0.404	0.136
8000	0.021	0.087	0.195	0.233	8000	0.046	0.088	0.241	0.119
16000	0.014	0.056	0.074	0.114	16000	0.041	0.071	0.078	0.098
32000	0.007	0.042	0.05	0.068	32000	0.036	0.023	0.085	0.0815

Appendix Table 4.110

Titration of IgG1 Showing mean OD values obtained in *F. hepatica* and *F. gigantica* infected sheep

Antigen (μg/ml)

F. hepatica.					*F. gigantic*				
FhGST	B	N	P1	P2	FhGST	B	N	P1	P2
2	0.288	0.530	0.445	0.670	2	0.02	0.52	0.79	1.095
1	0.259	0.580	0.508	0.745	1	0.019	0.37	0.863	0.63
0.5	0.354	0.594	0.681	0.936	0.5	0.022	0.192	0.35	0.41

Serum

F. hepatica.					*F. gigantic*				
Serum	B	N	P1	P2	Serum	B	N	P1	P2
50	0.0120	0.1580	0.6600	0.7800	50	0.012	0.2	0.8	0.695
100	0.0110	0.1320	0.5310	0.4300	100	0.019	0.137	0.51	0.63
200	0.0120	0.1090	0.2900	0.1800	200	0.028	0.2	0.35	0.41

Monoclonal antibody

F. hepatica.					*F. gigantic*				
McAb	B	N	P1	P2	McAb	B	N	P1	P2
10	0.120	0.272	0.452	0.341	10	0.12	0.1542	0.465	0.3095
20	0.011	0.142	0.310	0.253	20	0.109	0.169	0.509	0.263
40	0.010	0.061	0.230	0.136	40	0.026	0.079	0.372	0.245
80	0.009	0.056	0.209	0.087	80	0.033	0.049	0.211	0.134
160	0.008	0.055	0.127	0.101	160	0.04	0.021	0.135	0.121
320	0.007	0.103	0.090	0.027	320	0.047	0.014	0.09	0.16

Conjugate

F. hepatica.					*F. gigantic*				
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.012	0.158	0.690	0.680	1000	0.012	0.162	0.87	0.95
2000	0.011	0.082	0.310	0.530	2000	0.019	0.137	0.59	0.63
4000	0.010	0.060	0.300	0.360	4000	0.026	0.092	0.32	0.45
8000	0.009	0.050	0.239	0.210	8000	0.033	0.055	0.21	0.24
16000	0.008	0.031	0.127	0.220	16000	0.04	0.038	0.15	0.21
32000	0.007	0.026	0.090	0.140	32000	0.047	0.011	0.09	0.16

Appendix Table 4.111

Titrations of IgM Showing mean OD values obtained in *F. hepatica* and *F. gigantica* infected sheep.

Antigen (μg/ml)

F. hepatica.					*F. gigantic*				
FhGST	B	N	P1	P2	FhGST	B	N	P1	P2
2	0.106	0.295	0.872	0.642	2	0.087	0.387	0.483	0.527
1	0.084	0.264	0.887	0.594	1	0.081	0.288	0.614	0.668
0.5	0.076	0.211	0.614	0.541	0.5	0.064	0.316	0.414	0.451

Serum

F. hepatica.					*F. gigantic*				
Serum	B	N	P1	P2	Serum	B	N	P1	P2
50	0.016	0.532	0.830	0.829	50	0.020	0.549	0.771	0.744
100	0.013	0.345	0.764	0.590	100	0.014	0.534	0.705	0.680
200	0.004	0.369	0.389	0.305	200	0.007	0.221	0.264	0.299

Conjugate

F. hepatica.					*F. gigantic*				
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.179	0.318	0.913	0.709	1000	0.084	0.252	0.910	0.725
2000	0.159	0.229	0.510	0.398	2000	0.102	0.215	0.500	0.583
4000	0.119	0.218	0.421	0.259	4000	0.098	0.124	0.891	0.625
8000	0.083	0.201	0.336	0.215	8000	0.77	0.119	0.215	0.213

Appendix Table 4.112

Titrations of IgG2 Showing mean OD values obtained in *F. hepatica* and *F. gigantica* infected sheep.

Antigen (μg/ml)

F. hepatica.					*F. gigantic*				
FhGST	B	N	P1	P2	FhGST	B	N	P1	P2
2	0.084	0.091	0.091	0.108	2	0.041	0.032	0.049	0.044
1	0.081	0.070	0.101	0.066	1	0.039	0.032	0.045	0.042
0.5	0.079	0.080	0.089	0.088	0.5	0.031	0.027	0.041	0.027

Serum

F. hepatica.					*F. gigantic*				
Serum	B	N	P1	P2	Serum	B	N	P1	P2
50	0.008	0.012	0.016	0.011	50	0.015	0.009	0.010	0.020
100	0.008	0.009	0.018	0.006	100	0.014	0.010	0.011	0.015
200	0.008	0.008	0.010	0.007	200	0.011	0.017	0.018	0.009

Monoclonal antibody

F. hepatica.					*F. gigantic*				
McAb	B	N	P1	P2	McAb	B	N	P1	P2
10	0.008	0.012	0.110	0.150	10	0.009	0.011	0.014	0.020
20	0.008	0.009	0.016	0.016	20	0.011	0.014	0.013	0.018
40	0.008	0.008	0.014	0.017	40	0.012	0.012	0.012	0.019

Conjugate

F. hepatica.					*F. gigantic*				
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.031	0.033	0.060	0.095	1000	0.027	0.063	0.099	0.098
2000	0.028	0.030	0.050	0.035	2000	0.020	0.052	0.071	0.091
4000	0.022	0.025	0.022	0.031	4000	0.014	0.044	0.064	0.058
8000	0.020	0.026	0.027	0.033	8000	0.012	0.041	0.022	0.049

Appendix Table 4.113

Titrations of IgA Showing mean OD values obtained in *F. hepatica* and *F. gigantica* infected sheep.

Antigen (μg/ml)

F. hepatica.					*F. gigantic*				
FhGST	B	N	P1	P2	FhGST	B	N	P1	P2
2	0.066	0.058	0.050	0.034	2	0.031	0.047	0.053	0.032
1	0.047	0.041	0.058	0.044	1	0.039	0.041	0.046	0.050
0.5	0.048	0.040	0.024	0.029	0.5	0.038	0.028	0.060	0.032

Serum

F. hepatica.					*F. gigantic*				
Serum	B	N	P1	P2	Serum	B	N	P1	P2
50	0.020	0.014	0.020	0.018	50	0.027	0.021	0.023	0.021
100	0.017	0.019	0.021	0.022	100	0.014	0.017	0.013	0.032
200	0.020	0.015	0.017	0.016	200	0.022	0.020	0.024	0.030

Monoclonal antibody

F. hepatica.					*F. gigantic*				
McAb	B	N	P1	P2	McAb	B	N	P1	P2
10	0.012	0.013	0.022	0.028	10	0.022	0.023	0.024	0.024
20	0.017	0.018	0.023	0.022	20	0.016	0.027	0.013	0.022
40	0.019	0.015	0.017	0.016	40	0.022	0.021	0.024	0.023

Conjugate

F. hepatica.					*F. gigantic*				
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.031	0.033	0.060	0.095	1000	0.027	0.063	0.079	0.099
2000	0.028	0.030	0.050	0.085	2000	0.020	0.052	0.065	0.087
4000	0.022	0.025	0.022	0.031	4000	0.014	0.044	0.042	0.072
8000	0.020	0.026	0.027	0.033	8000	0.012	0.041	0.022	0.053
16000	0.029	0.030	0.020	0.031	16000	0.006	0.043	0.019	0.049
32000	0.011	0.031	0.030	0.029	32000	0.003	0.056	0.008	0.028

APPENDIX 4

Adjusted OD values for Antibody Responses to Glutathion S-Transferase (FhGST) By *F. hepatica* and *F. gigantica* infected sheep and cattle.

Experiment One (1): Adjusted values for *F. hepatica* (British and Peruvian strain) infected sheep and uninfected sheep

Appendix Table 4.114

Adjusted Total Ig OD values

WPI	SH. 5	SH. 6	SH. 7	SH. 9	SH. 10	SH. 8
-2	0.173	0.113	0.161	0.137	0.066	0.125
-1	0.181	0.108	0.101	0.164	0.016	0.006
0	0.179	0.029	0.174	0.176	0.168	0.030
1	0.160	0.181	0.104	0.206	0.128	0.062
2	0.154	0.167	0.109	0.200	0.131	0.048
3	0.091	0.125	0.054	0.219	0.308	0.082
4	0.264	0.375	0.158	0.227	0.393	0.125
5	0.265	0.333	0.161	0.225	0.293	0.135
6	0.124	0.444	0.176	0.247	0.245	0.125
7	0.246	0.645	0.160	0.139	0.245	0.146
8	0.247	0.444	0.248	0.278	0.405	0.194
9	0.121	0.401	0.446	0.167	0.768	0.195
10	0.198	0.339	0.553	0.244	0.288	0.055
11	0.307	0.909	0.439	0.353	0.504	0.176
12	0.108	0.888	0.406	0.154	0.415	0.177
13	0.172	0.547	0.422	0.218	0.405	0.125
14		0.399	0.135	0.307	0.245	0.135
15		0.717	0.122	0.425	0.484	0.209
16		0.242	0.160	0.050	0.333	0.059
17		0.514	0.169	0.022	0.305	0.174

Adjusted IgG_1 OD values

WPI	SH. 5	SH. 6	SH. 7	SH. 9	SH. 10	SH. 8
-2	0.072	0.063	0.023	0.141	0.027	0.028
-1	0.064	0.069	0.084	0.168	0.035	0.032
0	0.042	0.045	0.077	0.030	0.017	0.013
1	0.045	0.088	0.048	0.156	0.005	0.048
2	0.018	0.129	0.143	0.117	0.020	0.081
3	0.026	0.094	0.336	0.147	0.278	0.073
4	0.020	0.105	0.560	0.425	0.252	0.062
5	0.044	0.101	0.489	0.402	0.221	0.026
6	0.108	0.160	0.470	0.444	0.326	0.106
7	0.107	0.137	0.423	0.629	0.365	0.032
8	0.071	0.084	0.531	0.638	0.377	0.045
9	0.070	0.072	0.501	0.437	0.330	0.027
10	0.066	0.121	0.542	0.420	0.243	0.070
11	0.107	0.140	0.561	0.426	0.467	0.090
12	0.049	0.165	0.440	0.359	0.240	0.057
13	0.067	0.199	0.474	0.456	0.297	0.068
14		0.128	0.603	0.407	0.288	0.045
15		0.151	0.612	0.366	0.221	0.027
16		0.129	0.659	0.318	0.042	0.082
17		0.127	0.519	0.243	-0.017	0.080

Appendix Table 4.115

Adjusted IgM OD values

WPI	SH. 5	SH. 6	SH. 7	SH. 9	SH. 10	SH. 8
-2	0.229	0.145	0.322	0.111	0.216	0.188
-1	0.291	0.124	0.245	0.003	0.230	0.253
0	0.229	0.138	0.261	0.040	0.333	0.272
1	0.410	0.283	0.052	0.424	0.106	0.271
2	0.352	0.267	0.146	0.382	0.425	0.348
3	0.409	0.565	0.243	0.906	0.187	0.215
4	0.406	0.553	0.367	0.875	0.389	0.197
5	0.367	0.620	0.414	0.787	0.718	0.264
6	0.331	0.736	0.347	1.055	0.646	0.275
7	0.425	0.397	0.315	0.726	0.594	0.267
8	0.223	0.327	0.371	0.541	0.644	0.373
9	0.352	0.309	0.596	0.493	0.927	0.357
10	0.231	0.635	0.819	0.678	0.407	0.198
11	0.145	0.624	0.684	1.063	0.614	0.225
12	0.271	0.393	0.473	0.716	0.632	0.321
13	0.252	0.169	0.392	0.122	0.754	0.308
14		0.444	0.313	0.851	0.536	0.189
15		0.410	0.371	0.761	0.911	0.242
16		0.326	0.144	0.538	0.464	0.248
17		0.339	0.101	0.572	0.576	0.217

Adjusted IgG2 OD values

WPI	SH. 5	SH. 6	SH. 7	SH. 9	SH. 10	SH. 8
-2	0.062	0.078	0.038	0.045	0.046	0.058
-1	0.037	0.050	0.039	0.034	0.047	0.044
0	0.074	0.067	0.060	0.046	0.038	0.038
1	0.053	0.098	0.057	0.036	0.038	0.055
2	0.055	0.067	0.059	0.041	0.049	0.058
3	0.059	0.057	0.052	0.034	0.052	0.033
4	0.066	0.067	0.063	0.033	0.056	0.063
5	0.058	0.074	0.055	0.041	0.054	0.040
6	0.055	0.098	0.067	0.039	0.035	0.052
7	0.048	0.101	0.054	0.060	0.048	0.064
8	0.071	0.091	0.056	0.062	0.037	0.062
9	0.083	0.088	0.055	0.055	0.048	0.060
10	0.077	0.207	0.042	0.041	0.038	0.062
11	0.122	0.135	0.066	0.044	0.056	0.037
12	0.075	0.173	0.062	0.039	0.058	0.057
13	0.073	0.084	0.065	0.046	0.036	0.035
14		0.193	0.051	0.061	0.054	0.053
15		0.095	0.066	0.039	0.044	0.051
16		0.135	0.055	0.049	0.042	0.031
17		0.088	0.057	0.061	0.033	0.059

Appendix Table 4.116

Adjusted IgA OD values

WPI	SH. 5	SH. 6	SH. 7	SH. 9	SH. 10	SH. 8
-2	0.017	0.016	0.000	0.014	0.025	0.014
-1	0.022	0.015	0.003	0.016	0.019	0.017
0	0.041	0.017	0.014	0.010	0.019	0.013
1	0.041	0.028	0.006	0.034	0.022	0.012
2	0.032	0.040	0.024	0.055	0.039	0.011
3	0.045	0.017	0.014	0.042	0.020	0.014
4	0.026	0.019	0.035	0.047	0.019	0.014
5	0.017	0.022	0.022	0.042	0.021	0.018
6	0.019	0.028	0.022	0.060	0.021	0.022
7	0.023	0.021	0.021	0.053	0.032	0.023
8	0.018	0.023	0.019	0.061	0.024	0.016
9	0.016	0.018	0.024	0.080	0.030	0.019
10	0.016	0.020	0.026	0.075	0.037	0.022
11	0.013	0.035	0.023	0.060	0.050	0.020
12	0.027	0.039	0.015	0.039	0.046	0.016
13	0.033	0.033	0.015	0.065	0.059	0.021
14		0.036	0.026	0.080	0.045	0.019
15		0.039	0.032	0.035	0.045	0.022
16		0.027	0.035	0.048	0.033	0.019
17		0.032	0.028	0.057	0.027	0.026

Appendix Table 4.117

Experiment two (2): Adjusted values for *F. hepatica* (British strain) infected and uninfected sheep

Adjusted Total Ig OD values

WPI	SH. 24	SH. 26	SH. 28	SH. 30	SH. 22	SH. 32
-2	0.044	0.097	0.004	0.034	0.031	0.002
0	0.052	0.071	0.074	0.005	0.096	-0.028
2	0.258	0.169	0.165	0.019	0.100	0.098
4	0.522	0.331	0.790	0.299	0.036	0.077
6	0.502	0.191	0.597	0.189	0.105	0.050
8	0.668	0.243	0.472	0.252	0.091	0.002
9	0.715	0.754	0.403	0.375	0.131	0.059
10	0.722	0.255	0.361	0.249	0.113	0.083
11	0.699	0.331	0.351	0.237	0.095	0.035
12	0.532	0.158	0.487	0.431	0.070	0.108
13	0.826	0.261	0.259	0.321	0.129	0.116
14	0.944	0.102	0.240	0.237	0.132	0.115
15	1.038	0.130	0.318	0.206	0.178	0.104
16	0.637	0.031	0.578	0.377	0.103	0.117
17	0.698	0.121	0.380	0.360	0.030	0.102
18	0.665	0.043	0.431	0.162	0.134	0.101
19	0.563	0.242	0.184	0.073	0.156	0.033
20	0.367	0.120	0.124	0.197	0.107	0.081
21	0.471	0.064	0.413	0.146	0.087	0.001
22	0.615	0.069	0.373	0.124	0.159	0.064

Adjusted IgG1 OD values

WPI	SH. 24	SH. 26	SH. 28	SH. 30	SH. 22	SH. 32
-2	0.082	0.094	0.082	0.092	0.065	0.086
0	0.090	0.085	0.080	0.086	0.085	0.079
2	0.073	0.074	0.082	0.105	0.071	0.072
4	0.074	0.081	0.089	0.086	0.075	0.069
6	0.070	0.085	0.076	0.094	0.073	0.078
8	0.091	0.095	0.079	0.126	0.074	0.080
9	0.086	0.098	0.089	0.088	0.078	0.083
10	0.083	0.086	0.089	0.102	0.080	0.074
11	0.078	0.090	0.082	0.096	0.075	0.069
12	0.071	0.116	0.080	0.088	0.073	0.069
13	0.087	0.111	0.075	0.096	0.088	0.072
14	0.072	0.100	0.097	0.090	0.075	0.070
15	0.074	0.101	0.099	0.088	0.074	0.077
16	0.086	0.101	0.086	0.088	0.076	0.074
17	0.095	0.093	0.081	0.100	0.079	0.072
18	0.103	0.079	0.077	0.094	0.073	0.070
19	0.074	0.080	0.086	0.086	0.075	0.074
20	0.082	0.092	0.111	0.096	0.078	0.068
21	0.083	0.082	0.079	0.092	0.074	0.073
22	0.103	0.104	0.087	0.102	0.070	0.063

Appendix Table 4.118

Adjusted IgM OD values

WPI	SH. 24	SH. 26	SH. 28	SH. 30	SH. 22	SH. 32
-2	0.178	0.124	0.179	0.148	0.124	0.082
0	0.153	0.130	0.068	0.199	0.178	0.017
2	0.098	0.352	0.402	0.164	0.145	0.032
4	0.133	0.322	0.171	0.218	0.112	0.101
6	0.168	0.268	0.257	0.214	0.125	0.059
8	0.468	0.580	0.449	0.284	0.103	0.154
9	0.468	0.472	0.413	0.302	0.176	0.175
10	0.398	0.454	0.316	0.251	0.196	0.033
11	0.283	0.604	0.317	0.298	0.126	0.044
12	0.243	0.574	0.249	0.238	0.075	0.077
13	0.483	0.232	0.165	0.228	0.129	0.130
14	0.423	0.166	0.253	0.187	0.120	0.092
15	0.228	0.166	0.245	0.186	0.096	0.085
16	0.308	0.112	0.184	0.221	0.138	0.062
17	0.198	0.064	0.239	0.309	0.129	0.101
18	0.348	0.208	0.275	0.157	0.090	0.127
19	0.293	0.142	0.145	0.189	0.161	0.100
20	0.303	0.106	0.128	0.200	0.165	0.166
21	0.088	0.142	0.200	0.113	0.122	0.092
22	0.103	0.178	0.228	0.101	0.114	0.139

Adjusted IgG2 OD values

WPI	SH. 24	SH. 26	SH. 28	SH. 30	SH. 22	SH. 32
-2	0.041	0.032	0.049	0.044	0.042	0.045
0	0.039	0.032	0.045	0.042	0.042	0.045
2	0.031	0.027	0.041	0.027	0.044	0.029
4	0.036	0.026	0.036	0.046	0.038	0.033
6	0.031	0.026	0.054	0.044	0.030	0.031
8	0.033	0.029	0.048	0.047	0.038	0.039
9	0.029	0.027	0.035	0.029	0.047	0.046
10	0.028	0.025	0.035	0.046	0.030	0.034
11	0.033	0.029	0.054	0.041	0.029	0.035
12	0.028	0.026	0.045	0.033	0.029	0.046
13	0.031	0.028	0.059	0.040	0.037	0.037
14	0.031	0.028	0.040	0.041	0.041	0.045
15	0.030	0.027	0.059	0.047	0.046	0.029
16	0.025	0.025	0.053	0.040	0.036	0.040
17	0.028	0.028	0.056	0.047	0.038	0.035
18	0.031	0.029	0.049	0.040	0.033	0.032
19	0.030	0.031	0.056	0.037	0.029	0.039
20	0.030	0.033	0.035	0.030	0.034	0.033
21	0.028	0.028	0.059	0.032	0.042	0.038
22	0.023	0.033	0.062	0.034	0.033	0.038

Appendix Table 4.119

Adjusted IgA OD values

WPI	SH. 24	SH. 26	SH. 28	SH. 30	SH. 22	SH. 32
-2	0.044	0.044	0.052	0.033	0.026	0.035
0	0.036	0.040	0.041	0.044	0.023	0.022
2	0.031	0.038	0.033	0.049	0.023	0.028
4	0.036	0.042	0.038	0.049	0.042	0.021
6	0.044	0.027	0.036	0.029	0.022	0.027
8	0.042	0.042	0.051	0.033	0.034	0.040
9	0.042	0.033	0.026	0.045	0.039	0.033
10	0.028	0.029	0.029	0.045	0.028	0.024
11	0.045	0.028	0.023	0.035	0.037	0.025
12	0.024	0.042	0.044	0.051	0.029	0.024
13	0.043	0.034	0.038	0.028	0.038	0.023
14	0.025	0.029	0.045	0.038	0.038	0.029
15	0.045	0.024	0.025	0.036	0.035	0.039
16	0.039	0.040	0.025	0.030	0.042	0.027
17	0.043	0.036	0.023	0.039	0.039	0.042
18	0.024	0.029	0.026	0.054	0.038	0.032
19	0.036	0.044	0.041	0.046	0.041	0.033
20	0.039	0.037	0.046	0.051	0.032	0.032
21	0.033	0.046	0.045	0.054	0.031	0.036
22	0.038	0.024	0.025	0.036	0.035	0.039

Experiment three (3): Adjusted values for *F. gigantica* (Kenyan strain) infected and uninfected sheep

Appendix Table 4.120

Adjusted total Ig OD values

	Infected sheep							Uninfected sheep					
WPI	SH.11	SH.12	SH. 13	SH. 14	SH. 15	Mean (+)	SEM	SH.16	SH. 17	SH. 18	SH. 19	Mean (-)	SEM
-2	0.037	0.057	0.078	0.088	0.084	0.069	0.010	0.072	0.074	0.077	0.052	0.029	0.012
0	-0.021	0.152	0.072	0.127	0.255	0.117	0.046	0.00	0.046	0.049	0.024	0.044	0.008
2	0.432	0.196	0.024	0.475	0.259	0.277	0.082	0.025	0.056	0.060	0.035	0.048	0.008
4	0.348	0.239	0.126	0.350	0.202	0.253	0.043	0.032	0.059	0.062	0.037	0.046	0.008
6	0.285	0.186	0.132	0.302	0.367	0.254	0.042	0.028	0.058	0.061	0.036	0.062	0.006
8	0.482	0.225	0.085	0.246	0.431	0.294	0.072	0.060	0.069	0.072	0.047	0.031	0.012
9	0.278	0.244	0.153	0.336	0.516	0.305	0.060	0.000	0.047	0.051	0.025	0.095	0.011
10	0.318	0.260	0.077	0.409	0.236	0.260	0.055	0.123	0.092	0.095	0.070	0.015	0.016
11	0.328	0.307	0.193	0.340	0.514	0.336	0.052	-0.03	0.036	0.039	0.014	0.024	0.014
12	0.349	0.302	0.161	0.258	0.432	0.300	0.045	-0.01	0.043	0.046	0.021	0.065	0.006
13	0.272	0.115	0.077	0.136	0.476	0.215	0.073	0.066	0.071	0.075	0.049	0.034	0.011
14	0.247	0.108	0.109	0.316	0.354	0.227	0.051	0.006	0.049	0.053	0.028	0.039	0.010
15	0.255	0.167	0.329	0.321	0.236	0.262	0.030	0.016	0.053	0.056	0.031	0.054	0.007
16	0.200	0.156		0.313	0.264	0.233	0.014	0.044	0.063	0.067	0.041	0.094	0.010
17	0.099	0.116		0.266	0.278	0.190	0.014	0.120	0.091	0.094	0.069	0.079	0.007
18	0.081	0.150		0.310	0.264	0.201	0.012	0.091	0.081	0.084	0.059	0.095	0.011
19	0.251	0.023		0.535	0.246	0.264	0.017	0.123	0.092	0.095	0.070	0.087	0.009
20	0.170	0.045		0.207	0.244	0.167	0.013	0.107	0.086	0.090	0.064	0.021	0.014
21	0.243	0.017		0.189	0.105	0.139	0.013	-0.019	0.040	0.044	0.018	0.032	0.011
22	0.242	0.074		0.101	0.158	0.144	0.013	0.003	0.048	0.052	0.026	0.028	0.010

Appendix Table 4.121

Adjusted IgG1 OD values

	Infected sheep							Uninfected sheep					
WPI	SH.11	SH.12	SH. 13	SH. 14	SH. 15	Mean (+)	SEMean	SH.16	SH. 17	SH. 18	SH. 19	Mean (-)	SEM
-2	0.032	0.066	0.015	0.076	0.043	0.046	0.011	0.045	0.042	0.062	0.045	0.049	0.005
0	0.093	0.145	0.091	0.153	0.047	0.106	0.020	0.048	0.109	0.088	0.087	0.083	0.013
2	0.422	0.531	0.503	0.564	0.089	0.422	0.087	0.076	0.120	-0.001	0.018	0.053	0.028
4	0.652	0.719	0.790	0.851	0.103	0.623	0.134	0.085	0.066	-0.003	0.060	0.052	0.019
6	0.719	0.687	0.874	0.935	0.118	0.667	0.145	0.095	0.053	0.064	0.053	0.066	0.010
8	0.986	0.811	0.675	0.636	0.112	0.644	0.146	0.091	0.043	0.057	0.053	0.061	0.010
9	0.660	1.014	0.929	0.684	0.085	0.674	0.162	0.073	0.049	0.028	0.051	0.050	0.009
10	0.875	0.871	0.750	0.348	0.113	0.591	0.154	0.091	0.060	0.018	0.022	0.048	0.017
11	0.562	0.906	0.794	0.684	0.099	0.609	0.140	0.082	0.053	-0.008	-0.001	0.032	0.022
12	0.822	0.472	0.251	0.616	0.062	0.445	0.133	0.058	0.098	-0.070	0.051	0.034	0.036
13	0.517	0.777	0.633	0.561	0.093	0.516	0.115	0.078	0.086	0.064	0.043	0.068	0.009
14	0.343	0.761	0.613	0.585	0.112	0.483	0.114	0.091	0.072	0.044	0.051	0.065	0.011
15	0.506	0.497	0.283	0.641	0.102	0.406	0.095	0.084	0.051	0.017	0.028	0.045	0.015
16	0.416	0.533		0.471	0.124	0.386	0.091	0.099	0.101	-0.042	0.043	0.050	0.034
17	0.286	0.361		0.411	0.033	0.273	0.084	0.039	0.082	-0.048	0.029	0.026	0.027
18	0.263	0.530		0.595	0.123	0.378	0.111	0.098	0.078	-0.034	0.055	0.049	0.029
19	0.366	0.366		0.528	0.120	0.345	0.084	0.096	0.050	0.013	0.116	0.069	0.023
20	0.177	0.485		0.518	0.111	0.323	0.104	0.090	0.107	-0.046	0.100	0.063	0.036
21	0.152	0.384		0.386	0.029	0.238	0.089	0.036	0.062	-0.052	0.040	0.022	0.025
22	0.197	0.408		0.450	0.044	0.275	0.095	0.046	0.051	0.056	0.067	0.055	0.004

Appendix Table 4.122

Adjusted IgM OD values

	Infected sheep							Uninfected sheep					
WPI	SH.11	SH.12	SH. 13	SH. 14	SH. 15	TrMean	SEMean	SH.16	SH. 17	SH. 18	SH. 19	TrMean	SEM
-2	0.086	0.063	0.139	0.026	0.083	0.079	0.018	0.108	0.162	0.137	0.140	0.137	0.011
0	0.183	0.145	0.048	0.140	0.241	0.151	0.032	0.024	0.141	0.052	0.086	0.076	0.025
2	0.888	0.096	0.281	0.234	0.245	0.349	0.038	0.056	0.149	0.084	0.106	0.099	0.020
4	0.845	0.132	0.245	0.180	0.193	0.319	0.033	0.063	0.151	0.091	0.110	0.104	0.019
6	0.801	0.090	0.276	0.210	0.345	0.344	0.042	0.059	0.150	0.087	0.108	0.101	0.019
8	0.783	0.068	0.284	0.165	0.404	0.341	0.051	0.094	0.158	0.123	0.131	0.127	0.013
9	0.691	0.150	0.338	0.214	0.483	0.375	0.073	0.024	0.141	0.052	0.086	0.076	0.025
10	0.718	0.131	0.344	0.470	0.570	0.447	0.099	0.028	0.142	0.056	0.088	0.079	0.025
11	0.658	0.103	0.350	0.303	0.481	0.379	0.025	0.064	0.176	0.193	0.176	0.177	0.006
12	0.682	0.076	0.225	0.195	0.405	0.317	0.050	-0.01	0.133	0.021	0.065	0.053	0.031
13	0.630	0.112	0.131	0.275	0.446	0.319	0.082	0.014	0.138	0.042	0.079	0.068	0.027
14	0.572	0.113	0.071	0.148	0.333	0.247	0.070	0.101	0.160	0.130	0.135	0.132	0.012
15	0.479	0.166	0.099	0.188	0.224	0.231	0.065	0.035	0.144	0.063	0.092	0.091	0.022
16	0.464	0.105		0.189	0.250	0.252	0.078	0.045	0.146	0.073	0.099	0.114	0.016
17	0.305	0.088		0.086	0.263	0.186	0.058	0.077	0.154	0.105	0.119	0.175	0.006
18	0.418	0.006		0.126	0.250	0.200	0.079	0.161	0.175	0.189	0.173	0.152	0.008
19	0.649	-0.047		0.069	0.233	0.226	0.060	0.129	0.167	0.158	0.153	0.177	0.006
20	0.465	-0.017		0.090	0.231	0.192	0.040	0.164	0.176	0.193	0.176	0.164	0.006
21	0.568	-0.036		0.095	0.103	0.183	0.031	0.147	0.171	0.175	0.164	0.063	0.028
22	0.518	0.041		0.308	0.151	0.255	0.050	0.007	0.137	0.035	0.074	0.069	0.006

Appendix Table 4.123

Adjusted IgG2 OD values

	Infected sheep							Uninfected sheep					
WPI	SH.11	SH.12	SH. 13	SH. 14	SH. 15	TrMean	SEM	SH.16	SH. 17	SH. 18	SH. 19	TrMean	SEM
-2	0.081	0.106	0.022	0.169	0.081	0.051	0.024	0.093	0.081	0.065	0.063	0.040	0.019
0	0.093	0.081	0.035	0.079	0.128	0.043	0.015	0.010	0.085	0.082	0.058	0.059	0.017
2	0.110	0.089	0.017	0.024	0.129	0.050	0.023	0.00	0.073	0.073	0.048	0.048	0.018
4	0.074	0.076	0.029	0.006	0.184	0.074	0.031	0.034	0.109	0.101	0.076	0.080	0.017
6	0.046	0.071	0.002	0.091	0.165	0.075	0.027	0.003	0.078	0.076	0.052	0.052	0.018
8	0.037	0.084	-0.040	0.120	0.207	0.082	0.030	0.093	0.081	0.065	0.063	0.040	0.017
9	0.081	0.125	0.139	0.178	0.083	0.111	0.012	0.010	0.085	0.082	0.058	0.059	0.017
10	0.178	0.116	0.074	0.088	0.121	0.115	0.018	0.00	0.073	0.073	0.048	0.048	0.017
11	0.116	0.102	-0.067	0.155	0.129	0.087	0.030	0.025	0.100	0.094	0.070	0.072	0.017
12	0.217	0.111	0.083	0.162	0.168	0.148	0.023	0.028	0.103	0.096	0.072	0.074	0.017
13	0.152	0.142	0.092	0.115	0.166	0.133	0.013	0.016	0.091	0.087	0.063	0.064	0.017
14	0.114	0.137	0.034	0.113	0.194	0.118	0.026	0.045	0.120	0.110	0.085	0.090	0.017
15	0.100	0.086	0.073	0.066	0.136	0.092	0.012	0.029	0.104	0.097	0.073	0.076	0.017
16	0.044	0.119		-0.011	0.051	0.051	0.027	0.035	0.110	0.102	0.077	0.081	0.017
17	0.025	0.096		-0.050	0.233	0.076	0.030	0.026	0.101	0.095	0.071	0.073	0.017
18	0.040	0.094		-0.044	0.094	0.046	0.033	0.024	0.099	0.093	0.069	0.071	0.017
19	0.096	0.084		-0.026	0.132	0.072	0.034	0.003	0.078	0.076	0.052	0.052	0.018
20	0.062	0.111		-0.051	0.082	0.051	0.036	0.003	0.078	0.076	0.052	0.052	0.018
21	0.067	0.096		-0.023	0.031	0.043	0.026	0.004	0.079	0.077	0.053	0.053	0.018
22	0.088	0.099		-0.066	0.078	0.050	0.029	0.003	0.078	0.076	0.052	0.052	0.018

Appendix Table 4.124

Adjusted IgA OD values

	Infected sheep							Uninfected sheep					
WPI	SH.11	SH.12	SH. 13	SH. 14	SH. 15	Mean (+)	SEMean	SH.16	SH. 17	SH. 18	SH. 19	Mean (-)	SEM
-2	0.009	0.025	0.022	0.017	0.026	0.014	0.003	0.011	0.006	0.006	0.009	0.008	0.001
0	0.017	0.019	0.015	0.043	0.009	0.015	0.006	0.007	0.000	0.010	0.010	0.007	0.002
2	0.016	0.006	0.029	0.017	0.009	0.015	0.004	0.011	0.025	0.016	0.006	0.015	0.004
4	0.029	0.007	0.014	0.039	0.023	0.022	0.006	0.019	0.020	0.010	0.006	0.014	0.003
6	0.028	0.003	0.025	0.023	0.025	0.021	0.005	-0.01	0.023	0.013	0.013	0.010	0.007
8	0.021	0.004	0.033	0.010	0.005	0.015	0.006	-0.01	0.019	0.009	0.013	0.009	0.006
9	0.017	0.023	0.026	0.023	0.024	0.023	0.002	0.012	0.016	0.006	0.010	0.011	0.002
10	0.027	0.001	0.024	0.004	0.026	0.016	0.006	0.006	0.015	0.005	-0.001	0.006	0.003
11	0.026	0.027	0.022	0.004	0.004	0.017	0.005	0.014	0.004	-0.006	0.010	0.006	0.004
12	0.014	0.022	0.033	0.033	0.025	0.025	0.004	0.012	-0.010	0.000	0.011	0.003	0.005
13	0.019	0.006	0.016	0.017	0.023	0.016	0.003	0.016	0.004	-0.006	0.018	0.008	0.006
14	0.010	0.007	0.034	0.004	0.011	0.013	0.005	0.001	-0.005	-0.005	0.003	-0.002	0.002
15	0.010	0.006	0.042	0.012	0.028	0.020	0.007	0.010	0.012	0.002	0.011	0.009	0.002
16	0.006	0.002		0.025	0.015	0.012	0.005	0.007	0.006	0.016	0.014	0.011	0.003
17	0.021	0.021		0.039	0.017	0.025	0.005	-0.01	0.013	0.013	0.016	0.009	0.005
18	0.003	0.000		0.013	0.007	0.006	0.003	0.016	0.012	0.012	0.015	0.014	0.001
19	0.010	0.000		0.027	0.028	0.016	0.007	0.011	0.018	0.008	0.008	0.011	0.002
20	0.005	0.002		0.006	0.014	0.007	0.003	0.019	0.013	0.003	0.006	0.010	0.004
21	0.014	0.024		0.012	0.022	0.018	0.003	-0.01	0.000	0.010	0.007	0.003	0.003
22	0.015	0.010		0.021	0.028	0.011	0.065	0.006	0.004	0.014	0.010	0.009	0.002

Experiment Four (4): Adjusted values for *F. gigantica* (Kenyan strain) infected and uninfected sheep

Appendix Table 4.125

Adjusted total Ig OD values

WPI	SH.23	SH.25	SH. 27	SH. 29	SH. 21	SH.31
-2	0.023	0.020	0.081	0.053	0.036	0.003
0	0.018	0.053	-0.104	0.065	0.023	0.006
2	0.971	0.755	-0.072	0.190	0.049	0.172
4	0.834	0.639	0.141	0.167	0.038	-0.040
6	0.917	0.586	0.066	0.114	0.021	0.123
8	0.793	0.893	-0.033	0.514	0.204	0.131
9	0.636	0.701	-0.033	0.759	0.188	0.029
10	0.554	0.657	0.045	0.684	0.170	-0.047
11	0.552	0.620	0.096	0.306	-0.005	0.015
12	0.476	0.431	0.246	0.039	-0.122	-0.044
13	0.625	0.543	0.033	0.257	-0.040	-0.152
14	0.558	0.440	0.225	0.361	-0.018	0.033
15	0.557	0.380	0.699	0.251	-0.068	0.109
16		0.570	0.573	0.118	0.024	0.053
17			0.507	-0.016	0.090	-0.017
18			0.588	-0.021	0.135	0.109
19			1.128	0.133	0.047	-0.014
20			0.633	0.003	0.108	-0.082
21			1.413	-0.049	0.169	-0.056
22			0.873	-0.114	0.150	0.011

Adjusted IgG1 OD values

WPI	SH.23	SH.25	SH. 27	SH. 29	SH. 21	SH.31
-2	0.045	0.053	0.012	0.059	0.036	0.025
0	0.043	0.149	0.086	0.097	0.040	0.108
2	0.484	0.616	0.484	0.531	0.079	0.121
4	0.814	0.843	0.762	0.691	0.091	0.054
6	0.798	0.805	0.843	0.730	0.106	0.038
8	1.006	0.793	1.166	0.572	0.100	0.025
9	0.995	0.547	0.772	0.819	0.075	0.034
10	0.785	0.699	1.032	0.742	0.101	0.047
11	0.747	0.778	0.653	0.701	0.088	0.038
12	0.820	0.711	0.968	0.450	0.053	0.094
13	0.767	0.593	0.599	0.517	0.083	0.079
14	0.637	0.572	0.388	0.436	0.100	0.062
15	0.693	0.511	0.586	0.633	0.091	0.035
16		0.465	0.477	0.175	0.111	0.098
17			0.319	0.367	0.027	0.074
18			0.292	0.339	0.110	0.069
19			0.416	0.463	0.107	0.034
20			0.188	0.235	0.099	0.104
21			0.157	0.204	0.023	0.049
22			0.212	0.259	0.037	0.035

Appendix Table 4.126

Adjusted IgM OD values

WPI	SH.23	SH.25	SH. 27	SH. 29	SH. 21	SH.31
-2	0.137	0.161	0.159	0.130	0.043	0.076
0	-0.02	0.086	0.036	0.007	0.082	0.107
2	0.045	0.173	0.085	0.056	0.067	0.121
4	0.076	0.100	0.110	0.081	0.060	0.012
6	0.161	0.129	0.178	0.149	0.096	0.131
8	0.339	0.200	0.320	0.291	0.039	0.099
9	0.237	0.176	0.239	0.210	0.121	0.024
10	0.076	0.128	0.110	0.081	0.033	0.131
11	0.087	0.156	0.119	0.090	0.024	0.023
12	0.262	0.094	0.259	0.230	0.023	0.086
13	0.106	0.125	0.134	0.105	0.066	0.053
14	0.114	0.086	0.140	0.111	0.035	0.092
15	0.140	0.078	0.161	0.133	0.046	0.026
16		0.098	0.199	0.010	0.070	0.127
17			0.118	0.097	0.027	0.012
18			-0.011	0.004	0.035	0.094
19			0.021	0.007	0.016	0.058
20			0.046	0.134	0.040	0.064
21			-0.073	-0.001	0.053	0.107
22			0.006	0.078	0.043	0.076

Adjusted IgG2 OD values

WPI	SH.23	SH.25	SH. 27	SH. 29	SH. 21	SH.31
-2	0.055	0.051	0.003	0.033	0.052	0.001
0	0.011	0.047	0.014	0.073	0.041	0.051
2	0.059	0.067	0.059	0.058	0.058	0.031
4	0.035	0.010	0.054	0.070	0.012	0.010
6	0.034	0.006	0.051	0.063	-0.002	0.002
8	0.096	0.010	0.101	0.062	0.030	0.060
9	0.104	0.017	0.090	0.026	0.031	0.016
10	0.109	0.025	0.039	0.057	0.040	0.000
11	0.084	0.014	0.030	0.008	0.007	0.036
12	0.130	0.009	0.015	0.022	0.024	0.044
13	0.096	0.006	0.101	0.012	0.009	0.020
14	0.039	0.094	0.063	0.074	0.028	0.019
15	0.045	0.001	0.098	0.038	0.042	0.040
16	0.000	0.012	0.030	0.037	0.090	0.024
17		0.000	0.071	0.002	0.046	-0.01
18			0.077	0.027	0.040	0.023
19			0.072	0.101	0.023	0.037
20			0.056	0.086	0.010	0.000
21			0.140	0.106	0.012	0.053
22			0.056	0.071	0.043	0.002

Appendix Table 4.127

Adjusted IgA OD values

WPI	SH.23	SH.25	SH. 27	SH. 29	SH. 21	SH.31
-2	0.026	0.002	0.020	0.005	0.018	0.016
0	0.019	0.003	0.014	0.023	0.001	0.013
2	0.033	0.020	0.001	0.005	0.001	0.016
4	0.018	0.006	0.002	0.020	0.015	0.024
6	0.029	0.012	-0.002	0.009	0.017	-0.006
8	0.037	0.017	-0.001	0.001	-0.003	-0.002
9	0.030	0.014	0.018	0.009	0.016	0.017
10	0.028	0.015	-0.004	-0.004	0.018	0.011
11	0.026	0.015	0.022	-0.004	-0.004	0.019
12	0.037	0.018	0.017	0.016	0.017	0.017
13	0.020	0.022	0.001	0.005	0.015	0.021
14	0.038	0.023	0.002	-0.003	0.003	0.006
15	0.046	0.028	0.001	0.002	0.020	0.015
16		0.039	-0.003	0.011	0.007	0.013
17			0.016	0.020	0.009	-0.002
18			-0.005	0.003	-0.001	0.022
19			-0.005	0.012	0.020	0.016
20			-0.003	-0.002	0.006	0.024
21			0.019	0.002	0.014	0.000
22			-0.004	-0.005	0.020	0.012

Experiment 5 and 6: Calves infected with *F.hepatica* and *F. gigantica* respectively, Adjusted Antibody values in response to Fh-E/S and Fh-E/S

Appendix Table 4.128

Adjusted total Ig OD values

F. hepatica							F. gigantica				
WPI	14c	15c	23c	26c	34c	45c	WPI	Calf22	calf23	calf24	calf26
-2	0.029	0.032	0.071	0.026			-2	0.022	0.017	0.059	0.077
-1	0.051	0.026	0.07	0.016			-1	0.016	0.013	0.039	0.074
0	0.027	0.039	0.078	0.025			0	0.016	0.04	0.11	0.063
-24	0.069	0.021	0.086	0.031			1	0.023	0.089	0.03	0.077
-23	0.065	0.022	0.07	0.03			2	0.126	0.2	0.079	0.092
-22	0.073	0.014	0.057	0.018			3	0.185	0.63	0.053	0.009
-21	0.059	0.007	0.014	0.031			4	0.15	0.266	0.013	0.016
-20	0.077	0.013	0.019	0.035			5	0.134	0.419	0.131	0.001
-19	0.063	0.027	0.027	0.025			6	0.155	0.439	0.133	0.027
-18	0.073	0.034	0.078	0.024			7	0.274	0.296	0.179	0.038
-17	0.082	0.071	0.086	0.027			8	0.213	0.366	0.119	0.032
-16	0.093	0.05	0.05	0.014			9	0.315	0.276	0.144	0.047
-15	0.113	0.017	0.012	0.043			10	0.229	0.327	0.187	0.043
-14	0.126	0.04	0.056	0.018			11	0.164	0.503	0.299	0.069
-13	0.124	0.039	0.012	0.016			12	0.263	0.277	0.341	0.063
-12	0.089	0.044	0.041	0.025			13	0.354	0.574	0.431	0.024
-11	0.106	0.06	0.034	0.031			14	0.201	0.409	0.259	0.039
-10	0.098	0.047	0.049	0.03			15	0.176	0.456	0.286	0.028
-9	0.098	0.044	0.018	0.035			16	0.144	0.593	0.438	0.015
-8	0.136	0.042	0.013	0.025			17	0.258	0.392	0.44	0.005
-7	0.114	0.066	0.265	0.024			18	0.251	0.397	0.202	0.066
-6	0.112	0.087	0.27	0.037			19	0.391	0.289	0.187	0.055
-5	0.166	0.063	0.177	0.03			20	0.144	0.357	0.228	0.024
-4	0.138	0.054	0.2	0.04			21	0.258	0.705	0.193	0.041
-3	0.124	0.094	0.098	0.037			22	0.27	0.459	0.181	0.062
-2	0.161	0.123	0.157	0.03			23	0.341	0.234	0.194	0.03
-1	0.158	0.091	0.318	0.036			24	0.25	0.349	0.123	0.042
0	0.154	0.112	0.091	0.035	0.006	0.034	25	0.198	0.242	0.098	0.034
1	0.138	0.119	0.118	0.024	0.018	0.023	26	0.233	0.317	0.141	0.035
2	0.194	0.099	0.33	0.022	0.013	0.19	27	0.229	0.344	0.129	0.049
3	0.188	0.131	0.426	0.02	0.026	0.124	28	0.279	0.418	0.118	0.055
6	0.219	0.091	0.385	0.025	0.181	0.214	29	0.244	0.372	0.156	0.024
5	0.164	0.103	0.27	0.024	0.074	0.174	30	0.283	0.296	0.228	0.041
4	0.161	0.079	0.125	0.027	0.232	0.404	31	0.266	0.251	0.228	0.042
7	0.147	0.097	0.171	0.014	0.139	0.127					
8	0.205	0.183	0.126	0.024	0.187	0.121					
9	0.164	0.15	0.222		0.354	0.096					
10	0.31	0.161	0.26			0.143					
11	0.214	0.111	0.304								

Appendix Table 4.129
Adjusted IgG_1 OD values

F. hepatica							F. gigantica				
WPI	14c	15c	23c	26c	34c	45c	WPI	Calf22	calf23	calf24	calf26
-2	0.02	0.015	0.094	0.011							
-1	0.097	0.071	0.071	0.01			-2	0.063	0.002	0.009	0.021
0	0.178	0.081	0.119	0.006			-1	0.096	0.018	0.113	0.02
-24	0.064	0.22	0.077	0.052			0	0.16	0.031	0.151	0.016
-23	0.134	0.117	0.016	0.021			1	0.021	0.127	0.208	0.06
-22	0.022	0.385	0.131	0.075			2	0.168	0.085	0.171	0.03
-21	0.091	0.264	0.015	0.058			3	0.216	0.358	0.303	0.262
-20	0.031	0.284	0.025	0.145			4	0.356	0.401	0.271	0.066
-19	0.145	0.111	0.061	0.157			5	0.134	0.191	0.009	0.148
-18	0.14	0.204	0.021	0.075			6	0.069	0.312	0.086	0.159
-17	0.152	0.352	0.038	0.115			7	0.108	0.448	0.201	0.082
-16	0.244	0.146	0.073	0.064			8	0.268	0.283	0.035	0.12
-15	0.789	0.355	0.305	0.093			9	0.254	0.239	0.041	0.071
-14	0.192	0.366	0.274	0.031			10	0.307	0.437	0.326	0.099
-13	0.269	0.242	0.062	0.012			11	0.238	0.691	0.381	0.04
-12	0.258	0.227	0.14	0.08			12	0.246	0.961	0.336	0.022
-11	0.211	0.232	0.046	0.004			13	0.547	0.914	0.129	0.086
-10	0.289	0.52	0.242	0.05			14	0.438	1.156	0.18	0.014
-9	0.343	0.298	0.786	0.026			15	0.408	1.104	0.595	0.058
-8	0.356	0.686	0.419	0.025			16	0.282	1.172	0.255	0.035
-7	0.393	0.679	0.426	0.1			17	0.442	0.97	0.349	0.034
-6	0.353	0.289	0.822	0.102			18	0.567	0.896	0.209	0.105
-5	0.295	0.346	0.721	0.103			19	0.299	0.983	0.383	0.107
-4	0.234	0.333	0.463	0.088			20	0.476	1.172	0.59	0.108
-3	0.326	0.309	0.145	0.112			21	0.411	1.184	0.981	0.094
-2	0.681	0.379	0.255	0.058			22	0.327	0.836	0.659	0.117
-1	0.494	0.54	0.437	0.004			23	0.646	0.547	0.275	0.066
0	0.401	0.588	0.454	0.1	0.021	0.048	24	0.661	0.736	0.514	0.014
1	0.336	0.422	0.493	0.012	0.061	-0.003	25	0.622	0.568	0.71	0.105
2	0.356	0.66	0.274	0.117	0.127	-0.002	26	0.457	1.252	0.413	0.022
3	0.493	0.659	0.382	0.056	0.086	0.352	27	0.461	0.738	0.415	0.148
6	0.363	0.788	0.38	0.021	0.276	0.231	28	0.272	0.653	0.236	0.064
5	0.317	0.713	0.421	0.088	0.301	0.251	29	0.73	0.821	0.021	0.03
4	0.523	0.788	0.495	0.089	0.445	0.319	30	0.597	0.85	0.378	0.094
7	0.468	0.718	0.389	0.109	0.437	0.338	31	0.681	1.044	0.51	0.095
8	0.46	0.41	0.712		0.75	0.318	32	0.401	0.846	0.221	0.114
9	0.357	0.563	0.593		0.631	0.322					
10	0.383	0.768	0.566			0.409					
11	0.377	0.868	0.644								

Appendix Table 4.130

Adjusted IgM OD values

F. hepatica							F. gigantica				
WPI	14c	15c	23c	26c	34c	45c	WPI	Calf22	calf23	calf24	calf26
-2	0.231	0.078	0.215	0.064			-2	0.054	0.039	0.039	0.099
-1	0.244	0.028	0.146	0.05			-1	0.017	0.094	0.055	0.094
0	0.32	0.033	0.146	0.066			0	0.088	0.009	0.081	0.1
-24	0.231	0.305	0.238	0.005			1	0.171	0.133	0.185	0.077
-23	0.244	0.127	0.087	0.077			2	0.87	0.086	0.434	0.104
-22	0.32	0.258	0.081	0.163			3	0.448	0.175	0.444	0.136
-21	0.495	0.223	0.107	0.08			4	0.566	0.126	0.804	0.105
-20	0.439	0.273	0.088	0.239			5	0.569	0.284	0.683	0.165
-19	0.591	0.471	0.124	0.118			6	0.682	0.186	0.574	0.119
-18	0.659	0.513	0.081	0.014			7	0.507	0.139	0.518	0.08
-17	0.682	0.4	0.079	0.071			8	0.414	0.021	0.439	0.101
-16	0.844	0.525	0.238	0.217			9	0.5	0.019	0.596	0.156
-15	0.8	0.434	0.246	0.08			10	0.712	0.149	0.713	0.105
-14	0.862	0.458	0.228	0.102			11	0.542	0.271	0.566	0.113
-13	0.665	0.395	0.683	0.095			12	0.567	0.47	0.693	0.111
-12	0.635	0.434	0.73	0.111			13	0.696	0.241	0.814	0.117
-11	0.651	1.345	0.686	0.033			14	0.752	0.69	0.843	0.008
-10	0.692	0.883	0.808	0.059			15	0.434	0.283	0.451	0.097
-9	0.905	0.151	0.649	0.109			16	0.44	0.503	0.559	0.116
-8	0.943	0.244	0.59	0.055			17	0.574	0.548	0.686	0.095
-7	0.629	0.288	0.755	0.064			18	0.901	0.413	0.723	0.099
-6	0.879	0.146	0.607	0.01			19	0.686	0.525	0.579	0.078
-5	0.799	0.273	0.826	0.095			20	0.719	0.838	0.495	0.111
-4	0.751	0.153	0.922	0.066			21	0.74	0.543	0.725	0.1
-3	0.776	0.67	0.513	0.005			22	0.695	0.476	0.84	0.077
-2	0.346	0.343	0.609	0.077			23	0.803	0.344	0.698	0.104
-1	0.696	0.394	0.76	0.055			24	0.791	0.219	0.71	0.095
0	0.902	0.268	1.058	0.109	0.066	0.054	25	0.72	0.33	0.464	0.116
1	0.604	0.237	0.77	0.066	0.1	0.128	26	0.549	0.355	0.563	0.1
2	0.695	0.564	1	0.005	0.226	0.144	27	0.585	0.38	0.655	0.077
3	0.795	0.678	1.249	0.077	0.561	0.22	28	0.657	0.449	0.741	0.104
6	0.668	0.275	1.323	0.071	0.79	0.395	29	0.591	0.433	0.489	0.101
5	0.89	0.615	1.463	0.138	0.562	0.339	30	0.707	0.175	0.355	0.127
4	0.748	0.446	1.466	0.104	0.606	0.491	31	0.544	0.378	0.442	0.114
7	0.933	0.479	1.468	0.075	0.689	0.559	32	0.527	0.253	0.38	0.103
8	0.736	0.399	0.82		0.519	0.29					
9	0.884	0.376	0.656			0.532					
10	0.839	0.55	0.989			0.459					
11	0.69	0.46	0.835								

Appendix Table 4.131

Adjusted IgG2 OD values

F. hepatica							F. gigantica				
WPI	14c	15c	23c	26c	34c	45c	WPI	Calf22	calf23	calf24	calf26
-2	0.074	0.045	0.108	0.036			-2	0.068	0.043	0.039	0.053
-1	0.104	0.043	0.086	0.038			-1	0.085	0.069	0.093	0.055
0	0.081	0.051	0.069	0.072			0	0.076	0.067	0.117	0.089
-24	0.092	0.058	0.041	0.038			1	0.067	0.072	0.047	0.055
-23	0.077	0.027	0.03	0.046			2	0.043	0.075	0.123	0.063
-22	0.053	0.06	0.026	0.034			3	0.093	0.096	0.041	0.051
-21	0.043	0.025	0.021	0.042			4	0.054	0.05	0.053	0.059
-20	0.023	0.037	0.021	0.023			5	0.068	0.082	0.03	0.04
-19	0.048	0.03	0.004	0.063			6	0.039	0.086	0.007	0.08
-18	0.017	0.03	0.059	0.032			7	0.043	0.156	0.026	0.049
-17	0.048	0.035	0.053	0.054			8	0.037	0.076	0.038	0.071
-16	0.032	0.018	0.027	0.022			9	0.031	0.072	0.024	0.039
-15	0.014	0.062	0.013	0.047			10	0.022	0.11	0.027	0.064
-14	0.004	0.051	0.072	0.016			11	0.017	0.113	0.007	0.033
-13	0.063	0.038	0.061	0.05			12	0.054	0.178	0.05	0.067
-12	0.087	0.022	0.062	0.042			13	0.089	0.117	0.104	0.059
-11	0.023	0.053	0.05	0.028			14	0.039	0.127	0.053	0.045
-10	0.015	0.06	0.016	0.04			15	0.022	0.124	0.043	0.057
-9	0.032	0.054	0.097	0.009			16	0.022	0.131	0.038	0.026
-8	0.013	0.013	0.05	0.06			17	0.024	0.063	0.068	0.077
-7	0.052	0.058	0.126	0.048			18	0.041	0.086	0.038	0.065
-6	0.029	0.05	0.026	0.038			19	0.028	0.128	0.015	0.055
-5	0.018	0.027	0.035	0.038			20	0.028	0.088	0.048	0.055
-4	0.002	0.007	0.021	0.025			21	0.028	0.077	0.02	0.042
-3	0.088	0.043	0.009	0.04			22	0.052	0.047	0.016	0.057
-2	0.109	0.097	0.099	0.038			23	0.057	0.102	0.003	0.055
-1	0.067	0.043	0.035	0.036			24	0.033	0.036	0.104	0.053
0	0.036	0.022	0.055	0.028	0.054	0.044	25	0.028	0.049	0.048	0.045
1	0.035	0.042	0.037	0.038	0.065	0.051	26	0.017	0.025	0.02	0.055
2	0.03	0.258	0.058	0.019	0.05	0.02	27	0.033	0.112	0.016	0.036
3	0.096	0.102	0.124	0.071	0.026	0.053	28	0.05	0.068	0.018	0.088
6	0.029	0.145	0.102	0.064	0.016	0.018	29	0.076	0.062	0.02	0.081
5	0.038	0.095	0.373	0.038	0.01	0.03	30	0.03	0.06	0.016	0.055
4	0.056	0.198	0.256	0.036	0.026	0.044	31	0.056	0.15	0.021	0.053
7	0.073	0.183	0.145	0.037	0.016	0.051	32	0.033	0.121	0.02	0.054
8	0.137	0.141	0.122		0.024	0.02					
9	0.087	0.122	0.152		0.025	0.043					
10	0.078	0.177	0.157		0.015	0.05					
11	0.08	0.097	0.143			0.03					

Appendix Table 4.132

Adjusted IgA OD values

F. hepatica							F. gigantica				
WPI	14c	15c	23c	26c	34c	45c	WPI	Calf22	calf23	calf24	calf26
-2	0.037	0.031	0.043	0.036			-2	0.051	0.03	0.032	0.029
-1	0.041	0.045	0.044	0.044			-1	0.033	0.033	0.038	0.04
0	0.029	0.059	0.04	0.016			0	0.035	0.045	0.016	0.041
-24	0.07	0.069	0.053	0.04			1	0.023	0.05	0.035	0.029
-23	0.057	0.053	0.036	0.041			2	0.026	0.059	0.066	0.032
-22	0.042	0.041	0.032	0.034			3	0.019	0.043	0.03	0.024
-21	0.078	0.025	0.033	0.025			4	0.028	0.016	0.023	0.033
-20	0.023	0.01	0.04	0.033			5	0.017	0.021	0.029	0.022
-19	0.03	0.027	0.021	0.024			6	0.02	0.034	0.022	0.025
-18	0.011	0.034	0.031	0.034			7	0.029	0.027	0.03	0.035
-17	0.017	0.028	0.015	0.023			8	0.015	0.037	0.021	0.019
-16	0.017	0.025	0.032	0.03			9	0.017	0.021	0.027	0.022
-15	0.019	0.023	0.029	0.013			10	0.009	0.02	0.013	0.013
-14	0.005	0.026	0.063	0.034			11	0.01	0.017	0.03	0.014
-13	0.037	0.046	0.088	0.015			12	0.033	0.067	0.015	0.029
-12	0.053	0.031	0.064	0.046			13	0.006	0.048	0.04	0.032
-11	0.014	0.112	0.076	0.029			14	0.013	0.05	0.026	0.024
-10	0.013	0.019	0.073	0.04			15	0.013	0.054	0.035	0.017
-9	0.008	0.037	0.039	0.016			16	0.012	0.045	0.016	0.016
-8	0.023	0.027	0.042	0.019			17	0.016	0.038	0.018	0.021
-7	0.014	0.06	0.029	0.013			18	0.016	0.041	0.013	0.01
-6	0.01	0.045	0.037	0.023			19	0.016	0.046	0.021	0.033
-5	0.008	0.032	0.019	0.028			20	0.006	0.036	0.025	0.037
-4	0.006	0.022	0.06	0.019			21	0.007	0.028	0.018	0.011
-3	0.009	0.027	0.027	0.016			22	0.006	0.025	0.016	0.01
-2	0.026	0.042	0.048	0.028			23	0.028	0.032	0.025	0.021
-1	0.009	0.035	0.039	0.03			24	0.045	0.016	0.027	0.04
0	0.005	0.041	0.035	0.034	0.019	0.04	25	0.071	0.034	0.03	0.029
1	0.005	0.031	0.026	0.035	0.017	0.027	26	0.058	0.025	0.031	0.017
2	0.006	0.037	0.05	0.026	0.02	0.04	27	0.061	0.008	0.024	0.016
3	0.017	0.064	0.036	0.013	0.04	0.081	28	0.058	0.001	0.013	0.021
6	0.024	0.038	0.05	0.046	0.025	0.068	29	0.09	0.022	0.04	0.021
5	0.025	0.048	0.037	0.024	0.106	0.053	30	0.084	0.018	0.022	0.021
4	0.007	0.037	0.046	0.023	0.013	0.089	31	0.12	0.021	0.021	0.01
7	0.027	0.089	0.044	0.034	0.031	0.034	32	0.091	0.004	0.03	0.011
8	0.061	0.069	0.052		0.021	0.041					
9	0.093	0.087	0.049		0.054	0.022					
10	0.063	0.082	0.08			0.028					
11	0.05	0.067	0.094								

Appendix Table 4

Optical Density titration for ELISA using *Fasciola* spp. Excretory /Secretory products (FhES & FgES). Titrations of faecal total Ig Showing mean OD values from *F. hepatica* and *F. gigantica* infected sheep

Appendix Table 4.133

Antigen (μg/ml)

F. hepatica.					*F. gigantic*				
FhESP	B	N	P1	P2	FgESP	B	N	P1	P2
20	0.118	0.292	1.600	0.797	20	0.238	0.308	0.991	0.614
10	0.145	0.365	1.569	0.618	10	0.255	0.325	0.914	0.812
5	0.192	0.295	0.962	0.557	5	0.337	0.407	0.895	0.458
2.5	0.145	0.124	0.870	0.500	2.5	0.249	0.319	0.845	0.548
1.25	0.246	0.115	0.351	0.230	1.25	0.305	0.375	0.412	0.214
BBS	0.251	0.311	0.245	0.308	BBS	0.184	0.168	0.134	0.154

Conjugate

F. hepatica.					*F. gigantic*				
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.321	0.365	1.200	0.754	1000	0.312	0.325	0.914	0.812
2000	0.201	0.125	0.879	0.551	2000	0.207	0.235	0.689	0.511
4000	0.246	0.115	0.325	0.230	4000	0.137	0.245	0.246	0.347

Appendix Table 4.134

Titrations of faecal IgG_1 showing mean OD values from *F. hepatica* and *F. gigantica* infected sheep

Antigen (μg/ml)

F. hepatica.					*F. gigantic*				
FhESP	B	N	P1	P2	FgESP	B	N	P1	P2
20	0.081	0.087	0.112	0.162	20	0.016	0.019	0.045	0.026
10	0.088	0.096	0.14	0.186	10	0.021	0.021	0.051	0.018
5	0.106	0.111	0.156	0.193	5	0.013	0.020	0.054	0.044
2.5	0.113	0.115	0.156	0.183	2.5	0.013	0.014	0.041	0.024
1.25	0.104	0.102	0.15	0.153	1.25	0.020	0.015	0.043	0.018
BBS	0.094	0.101	0.134	0.105	BBS	0.012	0.012	0.042	0.024

Monoclonal antibody

F. hepatica. *F. gigantic*

McAb	B	N	P1	P2	McAb	B	N	P1	P2
10	0.014	0.019	0.015	0.017	10	0.018	0.020	0.023	0.030
20	0.021	0.017	0.016	0.012	20	0.017	0.031	0.028	0.022
40	0.013	0.014	0.018	0.010	40	0.013	0.021	0.019	0.026

Conjugate

F. hepatica.					*F. gigantic*				
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.009	0.020	0.012	0.019	1000	0.014	0.028	0.025	0.025
2000	0.016	0.010	0.011	0.016	2000	0.012	0.031	0.024	0.030
4000	0.011	0.009	0.015	0.015	4000	0.012	0.025	0.027	0.023

Appendix Table 4.135

Titrations of faecal IgM showing mean OD values from *F. hepatica* and *F. gigantica* infected sheep

Antigen (μg/ml)

F. hepatica.					*F. gigantic*				
FhESP	B	N	P1	P2	FgESP	B	N	P1	P2
20	0.020	0.022	0.013	0.024	20	0.035	0.032	0.051	0.055
10	0.017	0.021	0.023	0.024	10	0.039	0.042	0.053	0.031
5	0.023	0.015	0.014	0.015	5	0.038	0.045	0.038	0.030
2.5	0.019	0.023	0.016	0.021	2.5	0.044	0.055	0.051	0.037
1.25	0.024	0.023	0.017	0.022	1.25	0.047	0.033	0.041	0.044
BBS	0.019	0.024	0.015	0.013	BBS	0.047	0.045	0.039	0.037

Conjugate

F. hepatica.					*F. gigantic*				
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.023	0.021	0.020	0.014	1000	0.033	0.045	0.039	0.035
2000	0.016	0.017	0.023	0.017	2000	0.040	0.050	0.044	0.043
4000	0.022	0.022	0.022	0.020	4000	0.031	0.042	0.040	0.044

Appendix Table 4.136

Titrations of faecal IgG_2 showing mean OD values
From *F. hepatica* and *F. gigantica* infected sheep

Antigen (µg/ml)

F. hepatica.					*F. gigantic*				
FhESP	B	N	P1	P2	FgESP	B	N	P1	P2
20	0.033	0.036	0.035	0.036	20	0.024	0.023	0.041	0.016
10	0.031	0.034	0.037	0.037	10	0.022	0.038	0.040	0.022
5	0.032	0.035	0.037	0.035	5	0.040	0.026	0.030	0.023
2.5	0.033	0.036	0.041	0.040	2.5	0.041	0.033	0.034	0.023
1.25	0.043	0.052	0.061	0.043	1.25	0.029	0.032	0.023	0.017
BBS	0.055	0.088	0.038	0.043	BBS	0.029	0.033	0.033	0.016

Monoclonal antibody

F. hepatica.					*F. gigantic*				
McAb	B	N	P1	P2	McAb	B	N	P1	P2
10	0.033	0.036	0.035	0.036	10	0.024	0.023	0.041	0.016
20	0.031	0.034	0.037	0.037	20	0.022	0.038	0.040	0.022
40	0.032	0.035	0.037	0.035	40	0.040	0.026	0.030	0.023

Conjugate

F. hepatica.					*F. gigantic*				
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.031	0.034	0.037	0.037	1000	0.034	0.023	0.041	0.016
2000	0.032	0.035	0.037	0.035	2000	0.023	0.017	0.040	0.022
4000	0.033	0.036	0.041	0.040	4000	0.033	0.016	0.030	0.023

Appendix Table 4.137

Titrations of faecal IgA showing mean OD values
from *F. hepatica* and *F. gigantica* infected sheep

Antigen (µg/ml)

F. hepatica.					*F. gigantic*				
FhESP	B	N	P1	P2	FgESP	B	N	P1	P2
20	0.340	0.365	0.966	0.472	20	0.087	0.057	0.990	0.615
10	0.134	0.122	0.814	0.314	10	0.079	0.058	0.810	0.590
5	0.116	0.106	0.714	0.359	5	0.091	0.041	0.675	0.490
2.5	0.102	0.125	0.411	0.168	2.5	0.119	0.078	0.141	0.138
1.25	0.073	0.098	0.124	0.102	1.25	0.115	0.049	0.060	0.185
BBS	0.071	0.090	0.085	0.065	BBS	0.193	0.148	0.176	0.152

Monoclonal antibody

F. hepatica.					*F. gigantic*				
McAb	B	N	P1	P2	McAb	B	N	P1	P2
10	0.423	0.248	0.812	0.418	10	0.124	0.049	0.701	0.510
20	0.119	0.167	0.786	0.402	20	0.151	0.058	0.673	0.421
40	0.104	0.221	0.321	0.138	40	0.187	0.068	0.247	0.37

Conjugate

F. hepatica.					*F. gigantic*				
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.235	0.314	0.679	0.528	1000	0.155	0.049	0.654	0.419
2000	0.216	0.111	0.312	0.267	2000	0.131	0.058	0.312	0.187
4000	0.143	0.086	0.241	0.165	4000	0.177	0.068	0.211	0.37

APPENDIX TABLE:4

Adjusted OD values For Faecal Antibody Responses to *Fasciola* spp. Excretory/Secretory Products (FhESP or FgESP) By *F. hepatica* and *F. gigantica infected sheep*

Experiment One (1): Adjusted values for *F. hepatica* (British and Peruvian strain) infected sheep and uninfected sheep

Appendix Table 4.138

Adjusted Total faecal Ig OD values / Adjusted faecal IgG1 OD values

WPI	SH5	SH6	SH7	SH8	SH9	SH10	WPI	SH5	SH6	SH7	SH8	SH9	SH10
-2	0.120	0.031	0.023	0.120	0.102	0.125	-2	0.003	0.003	0.010	0.006	0.008	0.017
-1	0.174	0.104	0.070	0.149	0.198	0.128	-1	0.007	0.005	0.012	0.010	0.008	0.012
0	0.268	0.034	0.126	0.104	0.126	0.170	0	0.006	0.011	0.017	0.006	0.008	0.018
1	0.616	0.125	0.117	0.126	0.220	0.149	1	0.002	0.006	0.008	0.008	0.012	0.007
2	0.626	0.118	0.110	0.149	0.468	0.215	2	0.006	0.002	0.013	0.008	0.013	0.009
3	0.586	0.559	0.551	0.098	0.159	0.242	3	0.001	0.006	0.014	0.009	0.004	0.007
4	0.744	0.450	0.826	0.091	0.333	0.147	4	0.003	0.009	0.014	0.007	0.002	0.015
5	0.608	0.949	0.606	0.055	0.374	0.404	5	0.001	0.011	0.013	0.010	0.004	0.016
6	0.614	0.374	0.886	0.112	0.225	0.560	6	0.001	0.008	0.016	0.006	0.008	0.007
7	1.016	0.206	1.066	0.098	0.468	0.207	7	0.002	0.007	0.006	0.004	0.008	0.021
8	0.946	0.799	0.649	0.154	0.206	0.275	8	0.004	0.008	0.008	0.008	0.002	0.011
9	0.918	0.308	0.875	0.087	0.463	0.117	9	0.003	0.009	0.008	0.004	0.006	0.012
10	0.890	0.179	0.171	0.107	0.206	0.269	10	0.000	0.012	0.005	0.006	0.009	0.017
11	0.704	0.276	0.814	0.201	0.319	0.162	11	0.006	0.008	0.005	0.007	0.014	0.012
12		0.090	0.082	0.100	0.341	0.087	12		0.010	0.012	0.009	0.014	0.005
13		0.204	0.196	0.164	0.163	0.360	13		0.012	0.008	0.008	0.002	0.010
14		0.215	0.207	0.107	0.385	0.500	14		0.006	0.005	0.011	0.007	0.018
15		0.109	0.101	0.085	0.644	0.492	15		0.004	0.004	0.008	0.008	0.008
16		0.055	0.047	0.080	0.550	0.288	16		0.008	0.004	0.010	0.002	0.012
17		0.043	0.035	0.107	0.237	0.302	17		0.003	0.008	0.011	0.003	0.009

Appendix Table 4.139

Adjusted faecal IgM OD values / Adjusted faecal IgG2 OD values

WPI	SH5	SH6	SH7	SH8	SH9	SH10	WPI	SH5	SH6	SH7	SH8	SH9	SH10
-2	0.012	0.009	0.005	0.009	0.009	0.010	-2	0.017	0.009	0.013	0.012	0.017	0.019
-1	0.009	0.008	0.015	0.009	0.015	0.005	-1	0.014	0.008	0.014	0.013	0.015	0.019
0	0.015	0.000	0.006	0.004	0.010	0.014	0	0.016	0.008	0.014	0.011	0.013	0.017
1	0.011	0.010	0.008	0.007	0.016	0.010	1	0.018	0.009	0.016	0.016	0.012	0.022
2	0.016	0.010	0.009	0.008	0.015	0.005	2	0.037	0.021	0.028	0.019	0.013	0.016
3	0.011	0.011	0.007	0.003	0.012	0.015	3	0.060	0.048	0.014	0.019	0.015	0.014
4	0.015	0.008	0.012	0.003	0.010	0.006	4	0.030	0.023	0.019	0.012	0.013	0.019
5	0.008	0.003	0.015	0.005	0.016	0.014	5	0.048	0.020	0.015	0.013	0.012	0.012
6	0.014	0.009	0.014	0.006	0.006	0.009	6	0.016	0.046	0.012	0.013	0.011	0.011
7	0.008	0.013	0.009	0.006	0.005	0.015	7	0.030	0.009	0.019	0.013	0.009	0.011
8	0.013	0.004	0.007	0.005	0.012	0.014	8	0.041	0.008	0.013	0.012	0.006	0.005
9	0.016	0.008	0.005	0.007	0.015	0.011	9	0.060	0.011	0.009	0.013	0.011	0.006
10	0.016	0.001	0.013	0.004	0.007	0.006	10	0.033	0.011	0.008	0.016	0.008	0.014
11	0.014	0.013	0.006	0.009	0.012	0.011	11	0.055	0.013	0.010	0.015	0.009	0.015
12	0.008	0.004	0.014	0.009	0.009	0.005	12		0.023	0.014	0.012	0.014	0.014
13	0.011	0.003	0.006	0.006	0.005	0.010	13		0.011	0.010	0.015	0.015	0.009
14	0.005	0.004	0.014	0.008	0.014	0.010	14		0.025	0.010	0.013	0.015	0.010
15	0.011	0.003	0.010	0.009	0.011	0.012	15		0.013	0.012	0.009	0.022	0.009
16	0.009	0.001	0.007	0.006	0.010	0.011	16		0.016	0.017	0.011	0.013	0.009
17	0.013	0.001	0.009	0.004	0.010	0.014	17		0.024	0.016	0.011	0.011	0.011

Appendix Table 4.140

Adjusted Faecal IgA OD values

WPI	SH5	SH6	SH7	SH8	SH9	SH10
-2	0.028	0.077	0.069	0.023	0.029	0.037
-1	0.024	0.065	0.059	0.059	0.173	0.053
0	0.021	0.082	0.087	0.017	0.210	0.055
1	0.043	0.137	0.120	0.039	0.520	0.069
2	0.069	0.134	0.150	0.069	0.669	0.125
3	0.133	0.909	0.162	0.037	0.344	0.132
4	0.079	0.443	0.181	0.000	0.532	0.201
5	0.070	0.415	0.191	0.046	0.332	0.147
6	0.140	0.242	0.171	0.049	0.583	0.224
7	0.085	0.224	0.121	0.003	0.274	0.233
8	0.033	0.124	0.098	0.018	0.232	0.183
9	0.078	0.101	0.118	-0.115	0.759	0.232
10	0.183	0.142	0.078	0.003	0.796	0.089
11	0.017	0.015	0.083	0.028	1.386	0.392
12		0.017	0.019	0.034	1.608	0.215
13		0.042	0.075	0.035	0.469	0.132
14		0.053	0.045	-0.012	1.186	0.196
15		0.006	0.001	0.057	1.744	0.226
16		0.009	0.523	0.055	0.500	0.014
17		-0.004	0.543	0.031	1.274	0.055

Appendix Table 4.141

Experiment two (3): Adjusted values for *F. gigantica* Kenyan strain) infected and uninfected sheep

Adjusted Total faecal Ig OD values

WPI	SH.11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)	SEM
-2	0.113	0.062	0.078	0.055	0.059	0.073	0.011	0.051	0.071	0.082	0.076	0.070	0.007
0	0.171	0.084	0.130	0.116	0.049	0.110	0.021	0.082	0.102	0.107	0.096	0.097	0.005
2	0.041	0.124	0.093	0.105	0.046	0.082	0.016	0.035	0.054	0.069	0.066	0.056	0.008
4	0.549	0.104	0.052	0.175	0.092	0.194	0.091	0.059	0.078	0.088	0.081	0.077	0.006
6	0.768	0.151	0.025	0.129	0.672	0.349	0.154	0.082	0.102	0.107	0.096	0.097	0.005
8	0.793	0.224	0.102	0.470	0.446	0.407	0.119	0.027	0.047	0.063	0.061	0.050	0.008
9	0.918	0.105	0.403	0.785	0.540	0.550	0.143	0.020	0.039	0.057	0.056	0.043	0.009
10	0.897	0.613	0.347	0.587	0.419	0.573	0.095	0.210	0.001	0.088	0.031	0.083	0.046
11	0.651	0.902	0.414	0.685	0.682	0.667	0.078	0.044	0.063	0.076	0.071	0.064	0.007
12	0.759	0.938	0.523	0.656	0.410	0.657	0.092	0.034	0.113	0.058	0.036	0.060	0.018
13	0.793	1.119	0.504	0.496	0.645	0.711	0.115	0.089	0.108	0.112	0.100	0.102	0.005
14	1.007	1.009	0.925	0.567	0.741	0.850	0.086	0.016	0.036	0.054	0.053	0.040	0.009
15	0.758	1.106	0.883	0.161	0.066	0.595	0.205	0.037	0.057	0.071	0.067	0.058	0.008
16	0.555	1.232	1.245	0.245	0.082	0.672	0.244	0.140	0.159	0.153	0.134	0.147	0.006
17	1.082	1.129	0.313	0.475	0.301	0.660	0.185	0.030	0.049	0.065	0.062	0.052	0.008
18	1.097	1.032	0.204	0.341	0.410	0.617	0.186	0.100	0.119	0.121	0.108	0.112	0.005
19	1.346	1.029	0.496	0.174	0.217	0.652	0.231	0.037	0.057	0.071	0.067	0.058	0.008
20	1.055	0.962		0.109	0.116	0.560	0.259	0.014	0.033	0.052	0.052	0.038	0.009
21	0.780	0.723		0.426	0.137	0.516	0.148	0.007	0.027	0.047	0.048	0.032	0.010
22	0.741	0.806		0.120	0.117	0.446	0.190	0.037	0.057	0.071	0.067	0.058	0.008

Appendix Table 4.142

Adjusted faecal IgG1 OD values

WPI	SH.11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)	SEM
-2	0.032	0.024	0.019	0.008	0.012	0.019	0.004	0.006	0.018	0.005	0.023	0.013	0.004
0	0.030	0.030	0.009	0.000	0.010	0.016	0.006	0.021	0.024	0.007	0.005	0.014	0.005
2	0.020	0.033	0.041	0.009	-0.004	0.020	0.008	-0.003	0.021	0.018	0.027	0.016	0.007
4	0.014	0.020	0.016	0.008	-0.004	0.011	0.004	-0.003	0.003	0.027	0.020	0.012	0.007
6	0.014	0.022	0.009	0.000	-0.010	0.007	0.006	0.018	0.006	0.007	0.005	0.009	0.003
8	0.021	0.021	0.016	0.005	-0.010	0.011	0.006	-0.006	-0.003	0.014	0.018	0.006	0.006
9	0.017	0.023	0.018	0.001	-0.010	0.010	0.006	-0.003	0.009	0.020	0.005	0.008	0.005
10	0.018	0.021	0.018	0.004	0.010	0.014	0.003	0.003	0.012	0.018	0.007	0.010	0.003
11	0.010	0.027	0.018	0.013	0.006	0.015	0.004	0.006	-0.003	0.018	0.007	0.007	0.004
12	0.012	0.025	0.016	0.003	0.000	0.011	0.005	0.012	-0.009	0.018	0.011	0.008	0.006
13	0.015	0.023	0.040	-0.003	0.012	0.017	0.007	-0.006	0.018	0.016	0.018	0.012	0.006
14	0.017	0.027	0.014	0.006	-0.008	0.011	0.006	-0.009	0.009	0.018	0.018	0.009	0.005
15	0.011	0.024	0.011	0.000	-0.010	0.007	0.006	0.003	0.006	0.005	0.011	0.006	0.002
16	0.013	0.023		0.000	0.000	0.011	0.005	-0.009	0.012	0.014	0.027	0.011	0.005
17	0.017	0.031		0.003	-0.004	0.013	0.006	0.009	0.000	0.014	0.027	0.013	0.006
18	0.028	0.449		0.013	-0.008	0.010	0.006	0.021	-0.012	0.023	0.009	0.010	0.004
19	0.023	0.025		0.001	0.006	0.020	0.008	0.015	0.006	0.000	0.011	0.008	0.003
20	0.012	0.041		0.010	0.014	0.019	0.006	0.018	-0.006	0.011	0.025	0.012	0.007
21	0.017	0.025		0.004	0.014	0.018	0.005	-0.003	0.009	0.027	0.007	0.010	0.006
22	0.013	0.023		0.005	0.000	0.013	0.005	0.018	0.003	0.009	0.005	0.009	0.003

Appendix Table 4.143

Adjusted faecal IgM OD values

WPI	SH.11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)	SEM
-2	0.028	0.022	0.022	0.028	0.025	0.025	0.001	0.020	0.028	0.019	0.015	0.021	0.003
0	0.032	0.016	0.012	0.029	0.033	0.024	0.004	0.023	0.015	0.034	0.018	0.023	0.004
2	0.020	0.020	0.021	0.031	0.011	0.021	0.003	0.017	0.026	0.020	0.040	0.026	0.005
4	0.018	0.019	0.024	0.017	0.010	0.018	0.002	0.021	0.029	0.024	0.026	0.025	0.002
6	0.013	0.025	0.033	0.029	0.016	0.023	0.004	0.012	0.028	0.024	0.015	0.020	0.004
8	0.029	0.028	0.013	0.020	0.023	0.023	0.003	0.020	0.020	0.034	0.036	0.028	0.004
9	0.033	0.028	0.024	0.018	0.016	0.024	0.003	0.020	0.016	0.025	0.043	0.026	0.006
10	0.033	0.014	0.024	0.018	0.015	0.021	0.004	0.015	0.012	0.014	0.038	0.020	0.006
11	0.019	0.021	0.029	0.023	0.022	0.023	0.002	0.028	0.020	0.020	0.025	0.023	0.002
12	0.033	0.012	0.021	0.019	0.023	0.022	0.003	0.028	0.015	0.033	0.021	0.024	0.004
13	0.015	0.014	0.016	0.027	0.020	0.018	0.002	0.025	0.027	0.016	0.026	0.024	0.003
14	0.018	0.014	0.018	0.013	0.022	0.019	0.002	0.025	0.025	0.036	0.030	0.029	0.003
15	0.019	0.021	0.015	0.029	0.017	0.021	0.002	0.025	0.026	0.018	0.043	0.028	0.005
16	0.023	0.012		0.017	0.018	0.018	0.002	0.016	0.013	0.035	0.036	0.025	0.006
17	0.013	0.017		0.032	0.025	0.019	0.004	0.032	0.028	0.035	0.030	0.031	0.001
18	0.026	0.034		0.024	0.020	0.027	0.003	0.014	0.019	0.026	0.029	0.022	0.003
19	0.023	0.025		0.021	0.010	0.020	0.003	0.011	0.030	0.044	0.033	0.030	0.007
20	0.021	0.035		0.028	0.012	0.026	0.004	0.031	0.019	0.034	0.018	0.026	0.004
21	0.011	0.014		0.013	0.023	0.015	0.002	0.021	0.013	0.033	0.044	0.028	0.007
22	0.031	0.027		0.030	0.016	0.024	0.003	0.016	0.023	0.044	0.020	0.026	0.006

Appendix Table 4.144

Adjusted faecal IgG2 OD values

WPI	SH.11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)	SEM
-2	0.028	0.036	0.024	0.023	0.035	0.029	0.003	0.014	0.017	0.024	0.025	0.020	0.003
0	0.027	0.035	0.021	0.046	0.033	0.032	0.004	0.023	0.014	0.020	0.014	0.018	0.002
2	0.017	0.025	0.049	0.027	0.018	0.027	0.006	0.024	0.014	0.016	0.029	0.021	0.004
4	0.021	0.029	0.050	0.038	0.024	0.032	0.005	0.024	0.018	0.021	0.015	0.020	0.002
6	0.010	0.018	0.032	0.036	0.008	0.021	0.006	0.015	0.026	0.014	0.030	0.021	0.004
8	0.020	0.028	0.032	0.038	0.023	0.028	0.003	0.014	0.021	0.018	0.025	0.020	0.002
9	0.023	0.031	0.049	0.033	0.027	0.033	0.004	0.018	0.023	0.023	0.026	0.023	0.002
10	0.014	0.022	0.040	0.024	0.014	0.023	0.005	0.021	0.018	0.029	0.014	0.020	0.003
11	0.014	0.022	0.041	0.029	0.014	0.024	0.005	0.015	0.026	0.018	0.026	0.021	0.003
12	0.016	0.024	0.029	0.050	0.017	0.027	0.006	0.021	0.018	0.023	0.025	0.022	0.002
13	0.019	0.027	0.043	0.032	0.021	0.028	0.004	0.024	0.015	0.026	0.024	0.022	0.002
14	0.025	0.033	0.041	0.032	0.030	0.032	0.003	0.018	0.018	0.018	0.023	0.019	0.001
15	0.017	0.025	0.030	0.023	0.018	0.023	0.002	0.023	0.026	0.014	0.015	0.019	0.003
16	0.014	0.022	0.023	0.041	0.014	0.023	0.005	0.027	0.023	0.027	0.023	0.025	0.001
17	0.006	0.014	0.030	0.043	0.002	0.019	0.008	0.020	0.026	0.018	0.023	0.022	0.002
18	0.028	0.036	0.033	0.033	0.035	0.033	0.001	0.024	0.021	0.026	0.018	0.022	0.002
19	0.030	0.038	0.030	0.040	0.038	0.035	0.002	0.018	0.018	0.028	0.026	0.023	0.003
20	0.019	0.027	0.038	0.033	0.021	0.028	0.004	0.023	0.017	0.016	0.018	0.018	0.002
21	0.014	0.022	0.044	0.046	0.014	0.028	0.007	0.020	0.024	0.024	0.027	0.024	0.002
22	0.008	0.016	0.049	0.049	0.005	0.025	0.010	0.023	0.023	0.023	0.014	0.021	0.002

Appendix Table 4.145

Adjusted faecal IgA OD values

WPI	SH.11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)	SEM
-2	0.102	0.041	0.078	0.048	0.050	0.064	0.011	0.056	0.045	0.038	0.050	0.047	0.004
0	0.104	0.033	0.080	0.053	0.045	0.063	0.013	0.078	0.066	0.034	0.046	0.056	0.010
2	0.052	0.045	0.038	0.043	0.003	0.036	0.009	0.078	0.066	0.000	0.012	0.039	0.019
4	0.944	0.573	0.630	0.553	0.615	0.663	0.109	0.061	0.049	0.010	0.022	0.036	0.012
6	0.364	0.469	0.758	1.003	0.340	0.587	0.087	0.075	0.063	0.270	0.282	0.173	0.060
8	0.764	0.886	0.755	1.003	0.175	0.717	0.095	0.042	0.031	0.138	0.150	0.090	0.031
9	0.095	0.237	0.073	0.030	0.183	0.124	0.038	0.063	0.052	0.144	0.156	0.104	0.027
10	0.014	0.104	0.013	0.073	0.225	0.086	0.039	0.080	0.068	0.178	0.190	0.129	0.032
11	0.130	0.144	0.055	0.118	0.173	0.124	0.020	0.078	0.066	0.136	0.148	0.107	0.021
12	0.018	0.073	0.225	0.003	0.173	0.098	0.044	0.061	0.049	0.136	0.148	0.099	0.025
13	0.241	0.292	0.440	0.540	0.225	0.348	0.050	0.045	0.033	0.178	0.190	0.112	0.042
14	0.008	0.139	1.005	0.025	0.078	0.251	0.190	0.014	0.002	0.060	0.072	0.037	0.017
15	0.061	0.306	0.278	-0.055	0.055	0.129	0.070	0.085	0.073	0.042	0.054	0.064	0.010
16	0.137	0.011	1.313	0.135	0.073	0.334	0.095	0.049	0.038	0.056	0.068	0.053	0.006
17	0.018	0.048	0.263	0.150	0.048	0.105	0.045	0.099	0.087	0.036	0.048	0.068	0.015
18	0.011	0.023		0.140	0.068	0.048	0.026	0.071	0.059	0.052	0.064	0.062	0.004
19	0.343	0.094		0.650	0.200	0.257	0.114	0.021	0.009	0.158	0.170	0.090	0.043
20	0.639	1.430		1.390	0.500	0.792	0.044	0.073	0.061	0.238	0.250	0.156	0.051
21	0.302	0.155		0.548	0.248	0.251	0.090	0.061	0.049	0.196	0.208	0.129	0.043
22	0.047	0.047		0.178	0.195	0.093	0.039	0.000	-0.012	0.154	0.166	0.077	0.048

Appendix Table 4

Optical Density titration for ELISA using
F. hepatica Cathepsin L1 protease
Titrations of faecal total Ig Showing mean OD values
from *F. hepatica* and *F. gigantica* infected sheep

Appendix Table 4.146

Antigen (mg/ml)

F. hepatica.					*F. gigantic*				
CAthepsin	B	N	PI	P2	Athepsi	B	N	PI	P2
2	0.111	0.151	0.278	0.367	2	0.113	0.13	0.277	0.177
1	0.087	0.131	0.495	0.230	1	0.092	0.109	0.223	0.156
0.5	0.14	0.141	0.252	0.298	0.5	0.120	0.117	0.232	0.212

Conjugate

F. hepatica.					*F. gigantic*				
Conj.	B	N	PI	P2	Conj.	B	N	PI	P2
1000	0.042	0.1314	0.387	0.29	1000	0.135	0.114	0.355	0.131
2000	0.035	0.1333	0.423	0.281	2000	0.136	0.115	0.287	0.236
4000	0.028	0.09	0.159	0.101	4000	0.103	0.075	0.1094	0.277

Appendix Table 4.147

Titrations of faecal IgG_1 showing mean OD values
from *F. hepatica* and *F. gigantica* infected sheep

Antigen (mg/ml)

F. hepatica.					*F. gigantic*				
CAthepsin	B	N	PI	P2	Athepsi	B	N	PI	P2
2	0.040	0.054	0.079	0.072	2	0.121	0.112	0.121	0.139
1	0.053	0.056	0.081	0.087	1	0.122	0.123	0.143	0.145
0.5	0.041	0.038	0.061	0.083	0.5	0.123	0.130	0.136	0.124

Monoclonal antibody

F. hepatica.					*F. gigantic*				
McAb	B	N	PI	P2	McAb	B	N	PI	P2
10	0.060	0.054	0.060	0.075	10	0.143	0.135	0.135	0.142
20	0.063	0.049	0.073	0.076	20	0.121	0.144	0.131	0.145
40	0.058	0.064	0.075	0.068	40	0.118	0.134	0.149	0.118

Conjugate

F. hepatica.					*F. gigantic*				
Conj.	B	N	PI	P2	Conj.	B	N	PI	P2
1000	0.050	0.059	0.073	0.069	1000	0.140	0.126	0.145	0.117
2000	0.053	0.037	0.084	0.077	2000	0.121	0.130	0.119	0.135
4000	0.036	0.061	0.060	0.057	4000	0.125	0.117	0.136	0.124

Appendix Table 4.148

Titrations of faecal IgM showing mean OD values
from *F. hepatica* and *F. gigantica* infected sheep

Antigen (mg/ml)

F. hepatica.					*F. gigantic*				
CAthepsin	B	N	PI	P2	Athepsi	B	N	PI	P2
2	0.018	0.013	0.014	0.016	2	0.047	0.052	0.055	0.085
1	0.011	0.011	0.018	0.013	1	0.051	0.048	0.056	0.058
0.5	0.013	0.008	0.011	0.014	0.5	0.063	0.043	0.066	0.095

Conjugate

F. hepatica.					*F. gigantic*				
Conj.	B	N	PI	P2	Conj.	B	N	PI	P2
1000	0.010	0.010	0.009	0.015	1000	0.060	0.064	0.060	0.062
2000	0.015	0.007	0.011	0.015	2000	0.040	0.056	0.076	0.089
4000	0.013	0.007	0.010	0.015	4000	0.058	0.060	0.090	0.055

Appendix Table 4.149

Titrations of faecal IgG_2 showing mean OD values

From *F. hepatica* and *F. gigantica* infected sheep

Antigen (mg/ml)

F. hepatica.					*F. gigantic*				
CAthepsin	B	N	P1	P2	Athepsi	B	N	P1	P2
2	0.0117	0.0161	0.0278	0.0367	2	0.025	0.031	0.032	0.039
1	0.0124	0.0131	0.495	0.0348	1	0.031	0.026	0.027	0.036
0.5	0.0105	0.0141	0.0252	0.0434	0.5	0.033	0.038	0.033	0.024

Monoclonal antibody

F. hepatica.					*F. gigantic*				
McAb	B	N	P1	P2	McAb	B	N	P1	P2
10	0.06	0.0492	0.0645	0.059	10	0.026	0.034	0.031	0.040
20	0.058	0.033	0.0423	0.0471	20	0.025	0.024	0.041	0.041
40	0.054	0.0265	0.0281	0.027	40	0.026	0.042	0.039	0.026

Conjugate

F. hepatica.					*F. gigantic*				
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.042	0.0392	0.0687	0.059	1000	0.027	0.042	0.038	0.037
2000	0.035	0.0333	0.0623	0.0471	2000	0.030	0.026	0.026	0.034
4000	0.028	0.0274	0.0359	0.0352	4000	0.026	0.028	0.038	0.035

Appendix Table 4.150

Titrations of faecal IgA showing mean OD values

from *F. hepatica* and *F. gigantica* infected sheep

Antigen (mg/ml)

F. hepatica.					*F. gigantic*				
CAthepsin	B	N	P1	P2	Athepsi	B	N	P1	P2
2	0.216	0.223	0.841	0.551	2	0.325	0.412	0.625	0.416
1	0.224	0.157	0.734	0.497	1	0.287	0.333	0.621	0.379
0.5	0.109	0.248	0.512	0.511	0.5	0.400	0.321	0.539	0.485

Monoclonal antibody

F. hepatica.					*F. gigantic*				
McAb	B	N	P1	P2	McAb	B	N	P1	P2
10	0.214	0.308	0.741	0.627	10	0.149	0.187	0.625	0.416
20	0.102	0.241	0.683	0.568	20	0.156	0.171	0.621	0.379
40	0.148	0.234	0.559	0.403	40	0.166	0.188	0.539	0.485

Conjugate

F. hepatica.					*F. gigantic*				
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.182	0.222	0.787	0.59	1000	0.215	0.185	0.795	0.57
2000	0.145	0.233	0.623	0.514	2000	0.241	0.169	0.67	0.453
4000	0.118	0.219	0.421	0.352	4000	0.216	0.215	0.404	0.136

Appendix Table 4

Adjusted OD values For Faecal Antibody Responses to *F. hepatica cathepsin L1 protease*
By *F. hepatica* and *F. gigantica infected sheep*

Experiment One (1): Adjusted values for *F. hepatica* (British and Peruvian strain) infected sheep and uninfected sheep

Appendix Table 4.151

Adjusted Total faecal Ig OD values / Adjusted faecal IgG1 OD values

WPI	SH5	SH6	SH7	SH8	SH9	SH10	WPI	SH5	SH6	SH7	SH8	SH9	SH10
-2	0.022	0.023	0.024	0.023	0.024	0.023	-2	0.017	0.018	0.020	0.016	0.017	0.037
-1	0.046	0.018	0.033	0.040	0.034	0.012	-1	0.020	0.003	0.031	0.024	0.032	0.021
0	0.052	0.045	0.132	0.045	0.020	0.068	0	0.003	0.019	0.007	0.004	0.020	0.030
1	0.080	0.118	0.066	0.045	0.065	0.006	1	0.008	0.012	0.027	0.028	0.037	0.021
2	0.219	0.256	0.233	0.065	0.174	0.255	2	0.017	0.005	0.034	0.012	0.035	0.022
3	0.088	0.229	0.068	0.031	0.224	0.312	3	0.013	0.017	0.012	0.010	0.013	0.025
4	0.151	0.172	0.177	0.026	0.242	0.245	4	0.009	0.020	0.010	0.033	0.031	0.016
5	0.155	0.248	0.206	0.043	0.169	0.265	5	0.027	0.014	0.007	0.022	0.033	0.021
6	0.229	0.446	0.357	0.046	0.303	0.346	6	0.030	0.011	0.020	0.023	0.020	0.012
7	0.235	0.236	0.390	0.044	0.160	0.507	7	0.025	0.021	0.022	0.015	0.018	0.021
8	0.225	0.346	0.236	0.072	0.235	0.217	8	0.018	0.003	0.025	0.023	0.034	0.031
9	0.246	0.235	0.186	0.068	0.160	0.112	9	0.006	0.007	0.021	0.013	0.019	0.015
10	0.219	0.238	0.138	0.026	0.162	0.221	10	0.006	0.010	0.029	0.006	0.034	0.030
11	0.162	0.199	0.164	0.033	0.135	0.195	11	0.007	0.014	0.026	0.019	0.018	0.023
12		0.260	0.038	0.058	0.177	0.193	12		0.016	0.028	0.034	0.039	0.021
13		0.311	0.147	0.055	0.211	0.266	13		0.003	0.008	0.030	0.023	0.027
14		0.151	0.045	0.023	0.103	0.483	14		0.015	0.027	0.009	0.027	0.015
15		0.301	0.011	0.037	0.205	0.335	15		0.013	0.011	0.016	0.018	0.019
16		0.156	0.026	0.039	0.106	0.210	16		0.015	0.020	0.037	0.014	0.034
17		0.138	0.060	0.031	0.094	0.071	17		0.003	0.027	0.020	0.014	0.014

Appendix Table 4.152

Adjusted faecal IgM OD values / Adjusted faecal IgG2 OD values

WPI	SH5	SH6	SH7	SH8	SH9	SH10	WPI	SH5	SH6	SH7	SH8	SH9	SH10
-2	0.020	0.012	0.014	0.016	0.014	-0.004	-2	0.029	0.021	0.028	0.013	0.017	0.016
-1	0.006	0.008	0.022	0.010	0.000	0.014	-1	0.021	0.022	0.016	0.022	0.016	0.024
0	0.010	0.002	0.008	0.012	0.004	0.006	0	0.016	0.027	0.024	0.013	0.024	0.013
1	0.010	0.010	0.008	0.002	0.008	0.010	1	0.017	0.029	0.020	0.021	0.024	0.014
2	0.004	0.006	0.004	0.014	-0.002	0.000	2	0.012	0.023	0.028	0.021	0.028	0.024
3	0.014	0.000	0.008	0.014	0.006	0.014	3	0.029	0.013	0.012	0.015	0.027	0.017
4	0.010	0.000	0.006	0.014	0.018	0.000	4	0.025	0.027	0.008	0.016	0.024	0.026
5	0.008	0.012	0.012	0.016	0.012	0.018	5	0.014	0.011	0.022	0.024	0.017	0.016
6	0.008	0.016	0.008	-0.004	0.006	0.004	6	0.028	0.017	0.027	0.027	0.020	0.017
7	0.016	0.004	-0.002	0.000	0.006	-0.002	7	0.020	0.009	0.015	0.026	0.021	0.022
8	0.002	0.002	0.002	0.004	0.006	0.000	8	0.011	0.025	0.018	0.021	0.017	0.012
9	0.016	0.016	0.008	0.000	0.004	0.016	9	0.024	0.020	0.013	0.011	0.013	0.024
10	0.014	0.010	0.014	0.018	0.004	0.006	10	0.021	0.010	0.027	0.024	0.027	0.019
11	0.012	0.014	0.002	0.002	0.016	0.012	11	0.019	0.012	0.011	0.014	0.028	0.024
12		0.020	0.012	0.004	0.004	0.010	12		0.018	0.027	0.017	0.026	0.013
13		0.000	0.008	0.014	0.002	0.008	13		0.027	0.013	0.022	0.024	0.018
14		0.004	0.016	0.018	0.012	0.002	14		0.010	0.031	0.021	0.011	0.020
15		0.002	0.020	0.010	0.002	0.012	15		0.024	0.024	0.026	0.028	0.027
16		0.008	0.012	-0.002	0.016	0.004	16		0.007	0.026	0.021	0.026	0.023
17		0.008	0.000	0.002	0.002	0.020	17		0.013	0.019	0.021	0.026	0.014

Appendix Table 4.153

Adjusted faecal IgA OD values

WPI	SH5	SH6	SH7	SH8	SH9	SH10
-2	0.033	0.020	0.038	0.035	0.039	0.068
-1	0.018	0.025	0.090	0.020	0.006	0.033
0	0.052	0.035	0.073	0.038	0.039	0.090
1	0.113	0.077	0.098	0.076	0.162	0.117
2	0.213	0.249	0.169	0.082	0.387	0.138
3	0.135	0.257	0.176	0.052	0.404	0.098
4	0.074	0.357	0.100	0.080	0.171	0.207
5	0.063	0.280	0.086	0.050	0.455	0.243
6	0.091	0.225	0.121	0.080	0.335	0.066
7	0.224	0.301	0.079	0.080	0.500	0.116
8	0.090	0.275	0.049	0.086	0.444	0.035
9	0.034	0.175	0.050	0.074	0.227	0.096
10	0.118	0.117	0.030	0.075	0.101	0.032
11	0.216	0.039	0.066	0.056	0.081	0.039
12		0.112	0.085	0.087	0.090	0.039
13		0.023	0.278	0.071	0.347	0.149
14		0.413	0.326	0.062	0.490	0.162
15		0.248	0.245	0.066	0.434	0.387
16		0.181	0.365	0.057	0.240	0.404
17		0.044	0.134	0.061	0.092	0.257

Experiment three (3): Adjusted values for *F. gigantica* (Kenyan strain) infected and uninfected sheep

Appendix Table 4.154

Adjusted Total faecal Ig OD values

WPI	SH.11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)	SEM
-2	0.113	0.062	0.078	0.055	0.059	0.073	0.011	0.051	0.071	0.082	0.076	0.070	0.007
0	0.171	0.084	0.130	0.116	0.049	0.110	0.021	0.082	0.102	0.107	0.096	0.097	0.005
2	0.041	0.124	0.093	0.105	0.046	0.082	0.016	0.035	0.054	0.069	0.066	0.056	0.008
4	0.549	0.104	0.052	0.175	0.092	0.194	0.091	0.059	0.078	0.088	0.081	0.077	0.006
6	0.768	0.151	0.025	0.129	0.672	0.349	0.154	0.082	0.102	0.107	0.096	0.097	0.005
8	0.793	0.224	0.102	0.470	0.446	0.407	0.119	0.027	0.047	0.063	0.061	0.050	0.008
9	0.918	0.105	0.403	0.785	0.540	0.550	0.143	0.020	0.039	0.057	0.056	0.043	0.009
10	0.897	0.613	0.347	0.587	0.419	0.573	0.095	0.210	0.001	0.088	0.031	0.083	0.046
11	0.651	0.902	0.414	0.685	0.682	0.667	0.078	0.044	0.063	0.076	0.071	0.064	0.007
12	0.759	0.938	0.523	0.656	0.410	0.657	0.092	0.034	0.113	0.058	0.036	0.060	0.018
13	0.793	1.119	0.504	0.496	0.645	0.711	0.115	0.089	0.108	0.112	0.100	0.102	0.005
14	1.007	1.009	0.925	0.567	0.741	0.850	0.086	0.016	0.036	0.054	0.053	0.040	0.009
15	0.758	1.106	0.883	0.161	0.066	0.595	0.205	0.037	0.057	0.071	0.067	0.058	0.008
16	0.555	1.232	1.245	0.245	0.082	0.672	0.244	0.140	0.159	0.153	0.134	0.147	0.006
17	1.082	1.129	0.313	0.475	0.301	0.660	0.185	0.030	0.049	0.065	0.062	0.052	0.008
18	1.097	1.032	0.204	0.341	0.410	0.617	0.186	0.100	0.119	0.121	0.108	0.112	0.005
19	1.346	1.029	0.496	0.174	0.217	0.652	0.231	0.037	0.057	0.071	0.067	0.058	0.008
20	1.055	0.962		0.109	0.116	0.560	0.259	0.014	0.033	0.052	0.052	0.038	0.009
21	0.780	0.723		0.426	0.137	0.516	0.148	0.007	0.027	0.047	0.048	0.032	0.010
22	0.741	0.806		0.120	0.117	0.446	0.190	0.037	0.057	0.071	0.067	0.058	0.008

Appendix Table 4.155

Adjusted faecal IgG1 OD values

WPI	SH.11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)	SEM
-2	0.032	0.024	0.019	0.008	0.012	0.019	0.004	0.006	0.018	0.005	0.023	0.013	0.004
0	0.030	0.030	0.009	0.000	0.010	0.016	0.006	0.021	0.024	0.007	0.005	0.014	0.005
2	0.020	0.033	0.041	0.009	-0.004	0.020	0.008	-0.003	0.021	0.018	0.027	0.016	0.007
4	0.014	0.020	0.016	0.008	-0.004	0.011	0.004	-0.003	0.003	0.027	0.020	0.012	0.007
6	0.014	0.022	0.009	0.000	-0.010	0.007	0.006	0.018	0.006	0.007	0.005	0.009	0.003
8	0.021	0.021	0.016	0.005	-0.010	0.011	0.006	-0.006	-0.003	0.014	0.018	0.006	0.006
9	0.017	0.023	0.018	0.001	-0.010	0.010	0.006	-0.003	0.009	0.020	0.005	0.008	0.005
10	0.018	0.021	0.018	0.004	0.010	0.014	0.003	0.003	0.012	0.018	0.007	0.010	0.003
11	0.010	0.027	0.018	0.013	0.006	0.015	0.004	0.006	-0.003	0.018	0.007	0.007	0.004
12	0.012	0.025	0.016	0.003	0.000	0.011	0.005	0.012	-0.009	0.018	0.011	0.008	0.006
13	0.015	0.023	0.040	-0.003	0.012	0.017	0.007	-0.006	0.018	0.016	0.018	0.012	0.006
14	0.017	0.027	0.014	0.006	-0.008	0.011	0.006	-0.009	0.009	0.018	0.018	0.009	0.005
15	0.011	0.024	0.011	0.000	-0.010	0.007	0.006	0.003	0.006	0.005	0.011	0.006	0.002
16	0.013	0.023	0.020	0.000	0.000	0.011	0.005	-0.009	0.012	0.014	0.027	0.011	0.005
17	0.017	0.031	0.016	0.003	-0.004	0.013	0.006	0.009	0.000	0.014	0.027	0.013	0.006
18	0.028	0.449	0.010	0.013	-0.008	0.010	0.006	0.021	-0.012	0.023	0.009	0.010	0.004
19	0.023	0.025	0.045	0.001	0.006	0.020	0.008	0.015	0.006	0.000	0.011	0.008	0.003
20	0.012	0.041	0.019	0.010	0.014	0.019	0.006	0.018	-0.006	0.011	0.025	0.012	0.007
21	0.017	0.025	0.030	0.004	0.014	0.018	0.005	-0.003	0.009	0.027	0.007	0.010	0.006
22	0.013	0.023	0.025	0.005	0.000	0.013	0.005	0.018	0.003	0.009	0.005	0.009	0.003

Appendix Table 4.156

Adjusted faecal IgM OD values

WPI	SH.11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)	SEM
-2	0.028	0.022	0.022	0.028	0.025	0.025	0.001	0.020	0.028	0.019	0.015	0.021	0.003
0	0.032	0.016	0.012	0.029	0.033	0.024	0.004	0.023	0.015	0.034	0.018	0.023	0.004
2	0.020	0.020	0.021	0.031	0.011	0.021	0.003	0.017	0.026	0.020	0.040	0.026	0.005
4	0.018	0.019	0.024	0.017	0.010	0.018	0.002	0.021	0.029	0.024	0.026	0.025	0.002
6	0.013	0.025	0.033	0.029	0.016	0.023	0.004	0.012	0.028	0.024	0.015	0.020	0.004
8	0.029	0.028	0.013	0.020	0.023	0.023	0.003	0.020	0.020	0.034	0.036	0.028	0.004
9	0.033	0.028	0.024	0.018	0.016	0.024	0.003	0.020	0.016	0.025	0.043	0.026	0.006
10	0.033	0.014	0.024	0.018	0.015	0.021	0.004	0.015	0.012	0.014	0.038	0.020	0.006
11	0.019	0.021	0.029	0.023	0.022	0.023	0.002	0.028	0.020	0.020	0.025	0.023	0.002
12	0.033	0.012	0.021	0.019	0.023	0.022	0.003	0.028	0.015	0.033	0.021	0.024	0.004
13	0.015	0.014	0.016	0.027	0.020	0.018	0.002	0.025	0.027	0.016	0.026	0.024	0.003
14	0.018	0.014	0.026	0.013	0.022	0.019	0.002	0.025	0.025	0.036	0.030	0.029	0.003
15	0.019	0.021	0.021	0.029	0.017	0.021	0.002	0.025	0.026	0.018	0.043	0.028	0.005
16	0.023	0.012	0.021	0.017	0.018	0.018	0.002	0.016	0.013	0.035	0.036	0.025	0.006
17	0.013	0.017	0.010	0.032	0.025	0.019	0.004	0.032	0.028	0.035	0.030	0.031	0.001
18	0.026	0.034	0.033	0.024	0.020	0.027	0.003	0.014	0.019	0.026	0.029	0.022	0.003
19	0.023	0.025	0.022	0.021	0.010	0.020	0.003	0.011	0.030	0.044	0.033	0.030	0.007
20	0.021	0.035	0.032	0.028	0.012	0.026	0.004	0.031	0.019	0.034	0.018	0.026	0.004
21	0.011	0.014	0.013	0.013	0.023	0.015	0.002	0.021	0.013	0.033	0.044	0.028	0.007
22	0.031	0.027	0.018	0.030	0.016	0.024	0.003	0.016	0.023	0.044	0.020	0.026	0.006

Appendix Table4.157

Adjusted faecal IgG2 OD values

WPI	SH.11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)	SEM
-2	0.028	0.036	0.024	0.023	0.035	0.029	0.003	0.014	0.017	0.024	0.025	0.020	0.003
0	0.027	0.035	0.021	0.046	0.033	0.032	0.004	0.023	0.014	0.020	0.014	0.018	0.002
2	0.017	0.025	0.049	0.027	0.018	0.027	0.006	0.024	0.014	0.016	0.029	0.021	0.004
4	0.021	0.029	0.050	0.038	0.024	0.032	0.005	0.024	0.018	0.021	0.015	0.020	0.002
6	0.010	0.018	0.032	0.036	0.008	0.021	0.006	0.015	0.026	0.014	0.030	0.021	0.004
8	0.020	0.028	0.032	0.038	0.023	0.028	0.003	0.014	0.021	0.018	0.025	0.020	0.002
9	0.023	0.031	0.049	0.033	0.027	0.033	0.004	0.018	0.023	0.023	0.026	0.023	0.002
10	0.014	0.022	0.040	0.024	0.014	0.023	0.005	0.021	0.018	0.029	0.014	0.020	0.003
11	0.014	0.022	0.041	0.029	0.014	0.024	0.005	0.015	0.026	0.018	0.026	0.021	0.003
12	0.016	0.024	0.029	0.050	0.017	0.027	0.006	0.021	0.018	0.023	0.025	0.022	0.002
13	0.019	0.027	0.043	0.032	0.021	0.028	0.004	0.024	0.015	0.026	0.024	0.022	0.002
14	0.025	0.033	0.041	0.032	0.030	0.032	0.003	0.018	0.018	0.018	0.023	0.019	0.001
15	0.017	0.025	0.030	0.023	0.018	0.023	0.002	0.023	0.026	0.014	0.015	0.019	0.003
16	0.014	0.022	0.023	0.041	0.014	0.023	0.005	0.027	0.023	0.027	0.023	0.025	0.001
17	0.006	0.014	0.030	0.043	0.002	0.019	0.008	0.020	0.026	0.018	0.023	0.022	0.002
18	0.028	0.036	0.033	0.033	0.035	0.033	0.001	0.024	0.021	0.026	0.018	0.022	0.002
19	0.030	0.038	0.030	0.040	0.038	0.035	0.002	0.018	0.018	0.028	0.026	0.023	0.003
20	0.019	0.027	0.038	0.033	0.021	0.028	0.004	0.023	0.017	0.016	0.018	0.018	0.002
21	0.014	0.022	0.044	0.046	0.014	0.028	0.007	0.020	0.024	0.024	0.027	0.024	0.002
22	0.008	0.016	0.049	0.049	0.005	0.025	0.010	0.023	0.023	0.023	0.014	0.021	0.002

Appendix Table 4.158

Adjusted faecal IgA OD values

WPI	SH.11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	SEM	SH. 16	SH. 17	SH. 18	SH. 19	Mean (-)	SEM
-2	0.102	0.041	0.078	0.048	0.050	0.064	0.011	0.056	0.045	0.038	0.050	0.047	0.004
0	0.104	0.033	0.080	0.053	0.045	0.063	0.013	0.078	0.066	0.034	0.046	0.056	0.010
2	0.052	0.045	0.038	0.043	0.003	0.036	0.009	0.078	0.066	0.000	0.012	0.039	0.019
4	0.944	0.573	0.630	0.553	0.615	0.663	0.109	0.061	0.049	0.010	0.022	0.036	0.012
6	0.364	0.469	0.758	1.003	0.340	0.587	0.087	0.075	0.063	0.270	0.282	0.173	0.060
8	0.764	0.886	0.755	1.003	0.175	0.717	0.095	0.042	0.031	0.138	0.150	0.090	0.031
9	0.095	0.237	0.073	0.030	0.183	0.124	0.038	0.063	0.052	0.144	0.156	0.104	0.027
10	0.014	0.104	0.013	0.073	0.225	0.086	0.039	0.080	0.068	0.178	0.190	0.129	0.032
11	0.130	0.144	0.055	0.118	0.173	0.124	0.020	0.078	0.066	0.136	0.148	0.107	0.021
12	0.018	0.073	0.225	0.003	0.173	0.098	0.044	0.061	0.049	0.136	0.148	0.099	0.025
13	0.241	0.292	0.440	0.540	0.225	0.348	0.050	0.045	0.033	0.178	0.190	0.112	0.042
14	0.008	0.139	1.005	0.025	0.078	0.251	0.190	0.014	0.002	0.060	0.072	0.037	0.017
15	0.061	0.306	0.278	-0.055	0.055	0.129	0.070	0.085	0.073	0.042	0.054	0.064	0.010
16	0.137	0.011	1.313	0.135	0.073	0.334	0.095	0.049	0.038	0.056	0.068	0.053	0.006
17	0.018	0.048	0.263	0.150	0.048	0.105	0.045	0.099	0.087	0.036	0.048	0.068	0.015
18	0.011	0.023		0.140	0.068	0.048	0.026	0.071	0.059	0.052	0.064	0.062	0.004
19	0.343	0.094		0.650	0.200	0.257	0.114	0.021	0.009	0.158	0.170	0.090	0.043
20	0.639	1.430		1.390	0.500	0.792	0.044	0.073	0.061	0.238	0.250	0.156	0.051
21	0.302	0.155		0.548	0.248	0.251	0.090	0.061	0.049	0.196	0.208	0.129	0.043
22	0.047	0.047		0.178	0.195	0.093	0.039	0.000	-0.012	0.154	0.166	0.077	0.048

Appendix Table 4.159

Optical Density titration for ELISA using
F. hepatica Glutathion S-Transferase (FhGST)
Titrations of faecal total Ig Showing mean OD values
from *F. hepatica* and *F. gigantica* infected sheep

Antigen (μg/ml)

F. hepatica.					*F. gigantic*				
FhGST	B	N	P1	P2	FhGST	B	N	P1	P2
2	0.102	0.197	**0.425**	0.377	2	0.113	0.163	0.3	0.142
1	**0.195**	**0.101**	0.317	**0.259**	1	0.044	0.074	0.28	0.121
0 5	0.172	0.189	0.266	0.200	0.5	0.120	0.117	0.232	0.177

Conjugate

F. hepatica.					*F. gigantic*				
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.121	0.114	0.512	0.215	1000	0.087	0.142	0.289	0.147
2000	0.07	0.085	0.536	0.209	2000	0.051	0.082	0.26	0.126
4000	0.065	0.035	0.271	0.133	4000	0.033	0.089	0.181	0.103

Appendix Table 4.160

Titrations of faecal IgG_1 showing mean OD values
from *F. hepatica* and *F. gigantica* infected sheep

Antigen (μg/ml)

F. hepatica.					*F. gigantic*				
FhGST	B	N	P1	P2	FhGST	B	N	P1	P2
2	0.050	0.059	0.042	0.062	2	0.123	0.130	0.136	0.124
1	0.053	0.037	0.060	0.054	1	0.124	0.113	0.119	0.136
0.5	0.036	0.061	0.063	0.049	0.5	0.143	0.135	0.135	0.142

Monoclonal antibody

F. hepatica.					*F. gigantic*				
McAb	B	N	P1	P2	McAb	B	N	P1	P2
10	0.084	0.077	0.087	0.065	10	0.113	0.131	0.150	0.127
20	0.060	0.057	0.065	0.063	20	0.135	0.136	0.139	0.139
40	0.080	0.081	0.063	0.086	40	0.124	0.113	0.130	0.122

Conjugate

F. hepatica.					*F. gigantic*				
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.051	0.038	0.041	0.051	1000	0.121	0.144	0.131	0.145
2000	0.039	0.044	0.050	0.040	2000	0.118	0.134	0.149	0.118
4000	0.039	0.048	0.046	0.058	4000	0.148	0.143	0.132	0.129

Appendix Table 4.161 Titrations of faecal IgM showing mean OD values
from *F. hepatica* and *F. gigantica* infected sheep

Antigen (μg/ml)

F. hepatica.					*F. gigantic*				
FhGST	B	N	P1	P2	FhGST	B	N	P1	P2
2	0.065	0.063	0.075	0.068	2	0.060	0.064	0.043	0.051
1	0.063	0.086	0.078	0.076	1	0.046	0.047	0.054	0.059
0.5	0.060	0.075	0.074	0.066	0.5	0.038	0.032	0.032	0.032

Conjugate

F. hepatica.					*F. gigantic*				
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.050	0.040	0.058	0.064	1000	0.088	0.054	0.060	0.054
2000	0.046	0.058	0.051	0.038	2000	0.055	0.085	0.094	0.087
4000	0.042	0.062	0.039	0.044	4000	0.056	0.058	0.060	0.062

Appendix Table 4.162

Titrations of faecal IgG_2 showing mean OD values

From *F. hepatica* and *F. gigantica* infected sheep

Antigen (μg/ml)

F. hepatica.					*F. gigantic*				
FhGST	B	N	P1	P2	FhGST	B	N	P1	P2
2	0.058	0.050	0.041	0.052	2	0.025	0.031	0.032	0.039
1	0.050	0.051	0.058	0.042	1	0.031	0.026	0.027	0.036
0.5	0.045	0.056	0.054	0.056	0.5	0.033	0.038	0.033	0.024

Monoclonal antibody

F. hepatica.					*F. gigantic*				
McAb	B	N	P1	P2	McAb	B	N	P1	P2
10	0.049	0.069	0.037	0.062	10	0.037	0.026	0.039	0.027
20	0.057	0.068	0.051	0.073	20	0.025	0.033	0.040	0.036
40	0.041	0.061	0.056	0.076	40	0.035	0.033	0.029	0.022

Conjugate

F. hepatica.					*F. gigantic*				
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.044	0.075	0.056	0.072	1000	0.026	0.042	0.039	0.026
2000	0.047	0.069	0.040	0.060	2000	0.035	0.022	0.027	0.038
4000	0.042	0.056	0.056	0.063	4000	0.036	0.035	0.042	0.029

Appendix Table 4.163

Titrations of faecal IgA showing mean OD values

from *F. hepatica* and *F. gigantica* infected sheep

Antigen (μg/ml)

F. hepatica.					*F. gigantic*				
FhGST	B	N	P1	P2	FhGST	B	N	P1	P2
2	0.148	0.112	0.423	0.384	2	0.063	0.174	0.462	0.267
1	0.167	0.142	0.504	0.312	1	0.098	0.167	0.449	0.269
0.5	0.069	0.111	0.436	0.286	0.5	0.102	0.108	0.351	0.199

Monoclonal antibody

F. hepatica.					*F. gigantic*				
McAb	B	N	P1	P2	McAb	B	N	P1	P2
10	0.146	0.166	0.286	0.221	10	0.065	0.124	0.385	0.197
20	0.410	0.142	0.305	0.209	20	0.075	0.116	0.397	0.263
40	0.132	0.133	0.214	0.135	40	0.340	0.084	0.265	0.21

Conjugate

F. hepatica.					*F. gigantic*				
Conj.	B	N	P1	P2	Conj.	B	N	P1	P2
1000	0.121	0.125	0.327	0.214	1000	0.098	0.142	0.695	0.457
2000	0.147	0.181	0.365	0.251	2000	0.105	0.204	0.362	0.422
4000	0.136	0.104	0.219	0.134	4000	0.114	0.185	0.304	0.136

Appendix Table 4.164

Adjusted OD values For Faecal Antibody Responses to Glutathion S-Transferase (FhGST) by By F. hepatica and *F. gigantica* infected sheep

Experiment One (1): Adjusted values for *F. hepatica* (British and Peruvian strain) infected sheep and uninfected sheep

Adjusted Total Faecal Ig OD values

WPI	SH5	SH6	SH7	SH8	SH9	SH10
-2	0.101	0.102	0.195	0.172	0.105	0.148
-1	0.125	0.197	0.101	0.189	0.119	0.137
0	0.131	0.124	0.167	0.194	0.099	0.193
1	0.159	0.197	0.230	0.194	0.164	0.131
2	0.298	0.335	0.234	0.114	0.325	0.380
3	0.167	0.308	0.308	0.180	0.398	0.437
4	0.230	0.251	0.314	0.175	0.425	0.370
5	0.234	0.327	0.216	0.152	0.317	0.390
6	0.308	0.425	0.317	0.166	0.515	0.471
7	0.314	0.355	0.339	0.193	0.305	0.532
8	0.304	0.425	0.236	0.121	0.415	0.342
9	0.325	0.314	0.203	0.117	0.304	0.237
10	0.298	0.317	0.171	0.175	0.307	0.346
11	0.241	0.278	0.188	0.182	0.368	0.320
12		0.339	0.104	0.107	0.329	0.318
13		0.390	0.177	0.104	0.380	0.391
14		0.423	0.109	0.172	0.220	0.508
15		0.380	0.186	0.186	0.370	0.460
16		0.235	0.196	0.108	0.225	0.335
17		0.217	0.119	0.180	0.207	0.196

Adjusted Faecal IgG1 OD values

WPI	SH5	SH6	SH7	SH8	SH9	SH10
-2	0.050	0.059	0.073	0.069	0.028	0.048
-1	0.053	0.037	0.084	0.077	0.043	0.032
0	0.036	0.061	0.060	0.057	0.031	0.041
1	0.041	0.051	0.080	0.081	0.048	0.032
2	0.050	0.040	0.087	0.065	0.046	0.033
3	0.046	0.058	0.065	0.063	0.024	0.036
4	0.042	0.062	0.063	0.086	0.042	0.027
5	0.060	0.054	0.060	0.075	0.044	0.032
6	0.063	0.049	0.073	0.076	0.031	0.023
7	0.058	0.064	0.075	0.068	0.029	0.032
8	0.051	0.038	0.078	0.076	0.045	0.042
9	0.039	0.044	0.074	0.066	0.030	0.026
10	0.039	0.048	0.082	0.059	0.045	0.041
11	0.040	0.054	0.079	0.072	0.029	0.034
12	0.053	0.056	0.081	0.087	0.050	0.032
13	0.041	0.038	0.061	0.083	0.034	0.038
14		0.055	0.080	0.062	0.038	0.026
15		0.052	0.064	0.069	0.029	0.030
16		0.055	0.073	0.090	0.025	0.045
17		0.037	0.080	0.073	0.025	0.025

Appendix Table 4.165

Adjusted Faecal IgM OD values

WPI	SH5	SH6	SH7	SH8	SH9	SH10
-2	0.018	0.013	0.014	0.016	0.015	0.006
-1	0.011	0.011	0.018	0.013	0.008	0.015
0	0.013	0.008	0.011	0.014	0.010	0.011
1	0.013	0.012	0.011	0.009	0.012	0.013
2	0.010	0.010	0.009	0.015	0.007	0.008
3	0.015	0.007	0.011	0.015	0.011	0.015
4	0.013	0.007	0.010	0.015	0.017	0.008
5	0.012	0.013	0.013	0.016	0.014	0.017
6	0.012	0.015	0.011	0.006	0.011	0.010
7	0.016	0.009	0.006	0.008	0.011	0.007
8	0.009	0.008	0.008	0.010	0.011	0.008
9	0.016	0.015	0.011	0.008	0.010	0.016
10	0.015	0.012	0.014	0.017	0.010	0.011
11	0.014	0.014	0.008	0.009	0.016	0.014
12		0.017	0.013	0.010	0.010	0.013
13		0.007	0.011	0.015	0.009	0.012
14		0.009	0.015	0.017	0.014	0.009
15		0.008	0.017	0.013	0.009	0.014
16		0.011	0.013	0.007	0.016	0.010
17		0.011	0.007	0.009	0.009	0.018

Adjusted Faecal IgG2 OD values

WPI	SH5	SH6	SH7	SH8	SH9	SH10
-2	0.058	0.050	0.057	0.058	0.063	0.062
-1	0.050	0.051	0.045	0.070	0.062	0.073
0	0.045	0.056	0.053	0.058	0.072	0.059
1	0.046	0.058	0.049	0.069	0.072	0.060
2	0.041	0.052	0.057	0.068	0.077	0.073
3	0.058	0.042	0.041	0.061	0.076	0.064
4	0.054	0.056	0.037	0.062	0.073	0.075
5	0.043	0.040	0.051	0.073	0.064	0.062
6	0.057	0.046	0.056	0.076	0.067	0.064
7	0.049	0.038	0.044	0.075	0.069	0.070
8	0.040	0.054	0.047	0.069	0.063	0.057
9	0.053	0.049	0.042	0.056	0.058	0.072
10	0.050	0.039	0.056	0.072	0.076	0.066
11	0.048	0.041	0.040	0.060	0.077	0.073
12		0.047	0.056	0.063	0.075	0.059
13		0.056	0.042	0.070	0.072	0.065
14		0.039	0.060	0.069	0.056	0.067
15		0.053	0.053	0.075	0.078	0.076
16		0.036	0.055	0.068	0.075	0.071
17		0.042	0.048	0.069	0.075	0.060

Appendix Table 4.166

Adjusted Faecal IgA OD values

WPI	SH5	SH6	SH7	SH8	SH9	SH10
-2	0.067	0.110	0.171	0.108	0.107	0.126
-1	0.152	0.087	0.113	0.109	0.115	0.103
0	0.129	0.102	0.110	0.111	0.170	0.141
1	0.147	0.162	0.119	0.162	0.189	0.159
2	0.247	0.412	0.176	0.170	0.339	0.173
3	0.169	0.423	0.182	0.130	0.350	0.146
4	0.108	0.369	0.121	0.167	0.495	0.219
5	0.197	0.457	0.110	0.127	0.384	0.243
6	0.125	0.377	0.138	0.125	0.304	0.125
7	0.258	0.487	0.104	0.104	0.414	0.158
8	0.124	0.450	0.108	0.145	0.377	0.104
9	0.138	0.305	0.197	0.102	0.232	0.145
10	0.152	0.221	0.125	0.107	0.148	0.162
11	0.250	0.108	0.148	0.107	0.135	0.170
12	0.196	0.214	0.109	0.109	0.141	0.168
13		0.185	0.263	0.110	0.312	0.207
14		0.308	0.302	0.109	0.307	0.176
15		0.340	0.237	0.102	0.370	0.277
16		0.314	0.333	0.124	0.241	0.315
17		0.115	0.148	0.138	0.142	0.252

Appendix Table 4.167

Experiment three (3): Adjusted values for *F. gigantica* (Kenyan strain) infected and uninfected sheep

Adjusted total Faecal Ig OD values

WPI	SH.11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	SEM	SH. 16	SH. 17	SH. 18	SH. 19	MEAN(-)	SEM
-2	0.113	0.092	0.120	0.142	0.117	0.117	0.008	0.110	0.110	0.121	0.120	0.115	0.003
0	0.130	0.109	0.117	0.137	0.118	0.122	0.005	0.116	0.124	0.112	0.128	0.120	0.004
2	0.096	0.075	0.118	0.168	0.119	0.115	0.016	0.127	0.116	0.090	0.117	0.113	0.008
4	0.135	0.114	0.355	0.131	0.230	0.193	0.045	0.109	0.121	0.127	0.110	0.117	0.004
6	0.136	0.115	0.287	0.236	0.246	0.204	0.033	0.119	0.129	0.127	0.108	0.121	0.005
8	0.277	0.223	0.232	0.194	0.204	0.226	0.014	0.112	0.112	0.101	0.124	0.112	0.005
9	0.222	0.240	0.140	0.205	0.215	0.204	0.017	0.122	0.116	0.120	0.118	0.119	0.001
10	0.242	0.206	0.142	0.207	0.217	0.203	0.017	0.114	0.125	0.080	0.119	0.110	0.010
11	0.102	0.245	0.137	0.202	0.212	0.180	0.026	0.110	0.118	0.118	0.127	0.118	0.003
12	0.133	0.112	0.168	0.233	0.243	0.178	0.026	0.127	0.142	0.119	0.121	0.127	0.005
13	0.096	0.750	0.131	0.196	0.206	0.276	0.050	0.102	0.125	0.070	0.117	0.104	0.012
14	0.160	0.039	0.295	0.360	0.870	0.345	0.060	0.111	0.110	0.118	0.126	0.116	0.004
15	0.120	0.199	0.155	0.120	0.230	0.165	0.026	0.112	0.112	0.116	0.112	0.113	0.001
16	0.106	0.085	0.141	0.206	0.216	0.151	0.026	0.108	0.060	0.108	0.112	0.097	0.012
17	0.200	0.179	0.235	0.300	0.310	0.245	0.026	0.107	0.123	0.107	0.120	0.114	0.004
18	0.341	0.320	0.376	0.441	0.451	0.386	0.026	0.109	0.071	0.109	0.060	0.087	0.013
19	0.245	0.224	0.280	0.345	0.355	0.290	0.026	0.120	0.115	0.114	0.116	0.116	0.001
20	0.177	0.156	0.212	0.277	0.287	0.222	0.026	0.120	0.117	0.129	0.107	0.118	0.005
21	0.122	0.101	0.157	0.222	0.232	0.167	0.026	0.114	0.119	0.108	0.112	0.113	0.002
22	0.142	0.121	0.177	0.242	0.252	0.187	0.026	0.126	0.113	0.122	0.110	0.118	0.004

Appendix Table 4.168

Adjusted Faecal IgG1 OD values

WPI	SH.11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	SEM	SH. 16	SH. 17	SH. 18	SH. 19	MEAN(-)	SEM
-2	0.121	0.112	0.121	0.139	0.150	0.129	0.007	0.126	0.124	0.145	0.151	0.137	0.007
0	0.122	0.123	0.143	0.145	0.147	0.136	0.006	0.150	0.128	0.115	0.139	0.133	0.007
2	0.123	0.130	0.136	0.124	0.137	0.130	0.003	0.112	0.135	0.117	0.118	0.121	0.005
4	0.124	0.113	0.119	0.136	0.145	0.127	0.006	0.127	0.150	0.133	0.129	0.135	0.005
6	0.143	0.135	0.135	0.142	0.123	0.136	0.004	0.130	0.145	0.121	0.139	0.134	0.005
8	0.121	0.144	0.131	0.145	0.116	0.131	0.006	0.147	0.136	0.120	0.120	0.131	0.007
10	0.118	0.134	0.149	0.118	0.116	0.127	0.006	0.119	0.142	0.126	0.131	0.130	0.005
12	0.148	0.143	0.132	0.129	0.136	0.138	0.004	0.140	0.130	0.118	0.114	0.126	0.006
14	0.140	0.126	0.145	0.117	0.146	0.135	0.006	0.136	0.132	0.143	0.129	0.135	0.003
16	0.121	0.130	0.119	0.135	0.141	0.129	0.004	0.133	0.144	0.116	0.116	0.127	0.007
18	0.125	0.117	0.136	0.124	0.145	0.129	0.005	0.132	0.146	0.132	0.140	0.138	0.003
19	0.113	0.131	0.150	0.127	0.147	0.134	0.007	0.115	0.150	0.122	0.144	0.133	0.008
20	0.135	0.136	0.139	0.139	0.120	0.134	0.004	0.128	0.128	0.122	0.140	0.130	0.004
21	0.124	0.113	0.130	0.122	0.139	0.126	0.004	0.147	0.120	0.133	0.127	0.132	0.006
22	0.137	0.149	0.144	0.129	0.127	0.137	0.004	0.115	0.123	0.144	0.122	0.126	0.006
23	0.130	0.149	0.141	0.120	0.119	0.132	0.006	0.131	0.134	0.139	0.130	0.134	0.002
24	0.126	0.120	0.122	0.129	0.112	0.122	0.003	0.113	0.128	0.120	0.129	0.123	0.004
25	0.150	0.150	0.116	0.146	0.146	0.142	0.006	0.102	0.135	0.146	0.105	0.122	0.011
26	0.133	0.136	0.138	0.134	0.130	0.134	0.001	0.113	0.070	0.123	0.115	0.105	0.012
27	0.135	0.116	0.101	0.130	0.102	0.117	0.007	0.113	0.129	0.140	0.146	0.132	0.007

Appendix Table 4.169

Adjusted Faecal IgM OD values

WPI	SH.11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	SEM	SH. 16	SH. 17	SH. 18	SH. 19	MEAN(-)	SEM
-2	0.060	0.064	0.088	0.072	0.026	0.062	0.010	0.047	0.044	0.088	0.072	0.063	0.011
0	0.046	0.047	0.059	0.068	0.044	0.053	0.005	0.031	0.025	0.059	0.068	0.046	0.011
2	0.038	0.032	0.069	0.063	0.033	0.047	0.008	0.032	0.041	0.069	0.063	0.051	0.009
4	0.040	0.046	0.081	0.088	0.040	0.059	0.011	0.035	0.025	0.081	0.088	0.057	0.016
6	0.043	0.051	0.056	0.074	0.044	0.054	0.006	0.043	0.042	0.056	0.074	0.054	0.007
8	0.054	0.059	0.094	0.089	0.031	0.065	0.012	0.026	0.037	0.094	0.089	0.062	0.018
10	0.032	0.032	0.088	0.054	0.028	0.047	0.011	0.029	0.032	0.088	0.054	0.051	0.014
12	0.047	0.052	0.055	0.085	0.031	0.054	0.009	0.038	0.036	0.055	0.085	0.054	0.011
14	0.051	0.048	0.056	0.058	0.042	0.051	0.003	0.026	0.023	0.056	0.058	0.041	0.009
16	0.063	0.043	0.066	0.095	0.047	0.063	0.009	0.027	0.037	0.066	0.095	0.056	0.015
18	0.045	0.033	0.060	0.054	0.046	0.048	0.005	0.042	0.045	0.060	0.054	0.050	0.004
19	0.060	0.047	0.094	0.087	0.027	0.063	0.012	0.045	0.017	0.094	0.087	0.061	0.018
20	0.060	0.064	0.060	0.062	0.035	0.056	0.005	0.037	0.039	0.060	0.062	0.050	0.007
21	0.040	0.056	0.076	0.089	0.041	0.060	0.010	0.034	0.030	0.076	0.089	0.057	0.015
22	0.058	0.060	0.090	0.055	0.044	0.061	0.008	0.046	0.039	0.090	0.055	0.058	0.011
23	0.049	0.055	0.094	0.060	0.032	0.058	0.010	0.036	0.023	0.094	0.060	0.053	0.016
24	0.050	0.046	0.061	0.094	0.034	0.057	0.010	0.034	0.045	0.061	0.094	0.059	0.013
25	0.034	0.036	0.060	0.074	0.043	0.049	0.008	0.040	0.028	0.060	0.074	0.051	0.010
26	0.033	0.038	0.095	0.093	0.033	0.058	0.015	0.036	0.021	0.095	0.093	0.061	0.019
27	0.052	0.063	0.083	0.069	0.040	0.061	0.007	0.034	0.041	0.083	0.069	0.057	0.012

Appendix Table 4.170

Adjusted Faecal IgG2 OD values

WPI	SH.11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	SEM	SH. 16	SH. 17	SH. 18	SH. 19	MEAN(-)	SEM
-2	0.025	0.031	0.032	0.039	0.025	0.030	0.003	0.025	0.032	0.030	0.038	0.031	0.003
0	0.031	0.026	0.027	0.036	0.026	0.029	0.002	0.027	0.036	0.037	0.028	0.032	0.003
2	0.033	0.038	0.033	0.024	0.035	0.033	0.002	0.024	0.040	0.024	0.037	0.031	0.004
4	0.042	0.035	0.029	0.036	0.028	0.034	0.003	0.042	0.027	0.025	0.035	0.032	0.004
6	0.034	0.040	0.033	0.042	0.036	0.037	0.002	0.041	0.026	0.041	0.035	0.036	0.004
8	0.037	0.032	0.040	0.027	0.028	0.033	0.003	0.027	0.041	0.041	0.030	0.035	0.004
10	0.037	0.026	0.039	0.027	0.039	0.034	0.003	0.038	0.041	0.031	0.026	0.034	0.003
12	0.025	0.033	0.040	0.036	0.036	0.034	0.003	0.042	0.027	0.030	0.034	0.033	0.003
14	0.035	0.033	0.029	0.022	0.036	0.031	0.003	0.040	0.024	0.039	0.030	0.033	0.004
16	0.032	0.028	0.040	0.023	0.025	0.030	0.003	0.023	0.032	0.024	0.025	0.026	0.002
18	0.042	0.029	0.042	0.031	0.038	0.036	0.003	0.032	0.027	0.025	0.024	0.027	0.002
19	0.026	0.034	0.031	0.040	0.027	0.032	0.003	0.042	0.038	0.037	0.032	0.037	0.002
20	0.025	0.024	0.041	0.041	0.030	0.032	0.004	0.026	0.026	0.034	0.028	0.029	0.002
21	0.026	0.042	0.039	0.026	0.026	0.032	0.004	0.028	0.038	0.035	0.023	0.031	0.003
22	0.035	0.022	0.027	0.038	0.027	0.030	0.003	0.038	0.023	0.026	0.025	0.028	0.003
23	0.036	0.035	0.042	0.029	0.036	0.036	0.002	0.030	0.028	0.031	0.026	0.029	0.001
24	0.029	0.036	0.027	0.027	0.028	0.029	0.002	0.023	0.030	0.041	0.032	0.032	0.004
25	0.039	0.035	0.032	0.039	0.030	0.035	0.002	0.031	0.026	0.031	0.035	0.031	0.002
26	0.040	0.029	0.041	0.035	0.037	0.036	0.002	0.031	0.027	0.034	0.024	0.029	0.002
27	0.024	0.024	0.041	0.030	0.033	0.030	0.003	0.038	0.027	0.036	0.030	0.033	0.003

Appendix Table 4.171

Adjusted Faecal IgA OD values

WPI	SH.11	SH. 12	SH. 13	SH. 14	SH. 15	Mean (+)	SEM	SH. 16	SH. 17	SH. 18	SH. 19	MEAN(-)	SEM
-2	0.076	0.022	0.015	0.060	0.047	0.044	0.011	0.040	0.006	0.038	0.011	0.024	0.009
0	0.058	0.022	0.023	0.073	0.023	0.040	0.011	0.022	0.058	0.018	0.012	0.028	0.010
2	0.007	0.047	0.008	0.079	0.015	0.031	0.014	0.047	0.076	0.027	0.060	0.053	0.010
4	0.062	0.063	0.105	0.168	0.090	0.098	0.019	0.021	0.012	0.027	0.013	0.018	0.004
6	0.208	0.177	0.120	0.226	-0.004	0.145	0.042	0.055	0.047	0.031	0.079	0.053	0.010
8	0.077	0.206	0.127	0.007	0.036	0.091	0.035	0.007	0.023	0.018	0.027	0.019	0.004
10	0.309	0.275	0.106	0.339	0.055	0.217	0.057	0.070	0.015	0.031	0.030	0.037	0.012
12	0.032	0.036	0.085	0.295	0.107	0.111	0.048	0.040	0.000	0.006	0.055	0.025	0.013
14	0.167	-0.004	0.256	0.208	0.103	0.146	0.045	0.022	0.086	0.059	0.014	0.045	0.017
16	0.447	0.036	0.313	0.353	0.168	0.263	0.073	0.040	0.023	0.060	0.060	0.046	0.009
18	0.218	0.085		0.134	0.226	0.166	0.034	0.022	0.015	0.073	0.073	0.046	0.016
19	0.149	0.724		0.112	0.167	0.288	0.085	0.047	0.014	-0.021	0.079	0.030	0.022
20	0.252	0.474		0.158	0.002	0.222	0.099	0.023	0.060	0.053	0.068	0.051	0.010
21	0.351	0.137		0.206	0.023	0.179	0.069	0.051	0.007	0.032	0.023	0.028	0.009
22	0.218	0.339		0.137	0.010	0.176	0.069	0.089	0.040	0.022	0.039	0.048	0.014
23	0.112	0.295		0.137	0.120	0.166	0.043	0.027	0.094	0.022	0.118	0.065	0.024
24	0.158	0.208		0.090	0.040	0.124	0.037	0.086	0.045	-0.020	0.082	0.048	0.025
25	0.206	0.353		-0.003	0.022	0.145	0.084	0.005	0.010	0.101	0.051	0.042	0.022
26	0.090	0.134		0.015	0.047	0.072	0.026	0.016	0.085	0.007	0.089	0.049	0.022
27	0.090	0.143		0.010	0.023	0.067	0.031	0.014	-0.062	-0.005	0.109	0.014	0.036

Appendix Table 4.172

Experiment 5 : Calves infected with *F.hepatica* and *F. gigantica* respectively,

Adjusted faecal total Ig OD values: Poilt Test

	Fh-E/S				Fh-Cathepsin				Fh-GST			
WPI	14c	15c	23c	26c	14c	15c	23c	26c	14c	15c	23c	26c
-26	0.046	0.058	0.049	0.069	0.059	0.003	0.040	0.024	0.038	0.036	0.055	0.085
-25	0.041	0.052	0.057	0.068	0.059	0.003	0.023	0.032	0.026	0.023	0.056	0.058
-24	0.058	0.042	0.041	0.061	0.048	0.003	0.032	0.027	0.027	0.037	0.066	0.095
-23	0.054	0.056	0.037	0.062	0.052	0.003	0.042	0.038	0.042	0.045	0.060	0.054
-15	0.043	0.040	0.051	0.073	0.055	0.004	0.026	0.026	0.045	0.017	0.094	0.087
-12	0.057	0.046	0.056	0.076	0.059	0.004	0.028	0.038	0.037	0.039	0.060	0.062
-9	0.049	0.038	0.044	0.075	0.052	0.003	0.038	0.023	0.034	0.030	0.076	0.089
-6	0.040	0.054	0.047	0.069	0.057	0.002	0.030	0.028	0.046	0.039	0.090	0.055
-3	0.053	0.049	0.042	0.056	0.049	0.002	0.023	0.030	0.026	0.002	0.031	0.027
0	0.067	0.063	0.073	0.072	0.069	0.002	0.031	0.026	0.069	0.058	0.064	0.077
1	0.050	0.051	0.045	0.070	0.062	0.073	0.064	0.062	0.043	0.040	0.051	0.073
3	0.045	0.056	0.053	0.058	0.072	0.059	0.067	0.064	0.057	0.046	0.056	0.076
4	0.046	0.058	0.049	0.069	0.072	0.060	0.069	0.070	0.049	0.038	0.044	0.075
6	0.041	0.052	0.057	0.068	0.077	0.073	0.063	0.057	0.040	0.054	0.047	0.069
9	0.058	0.042	0.041	0.061	0.076	0.061	0.058	0.072	0.053	0.049	0.042	0.056
12	0.054	0.056	0.037	0.062	0.073	0.075	0.076	0.066	0.050	0.039	0.056	0.072